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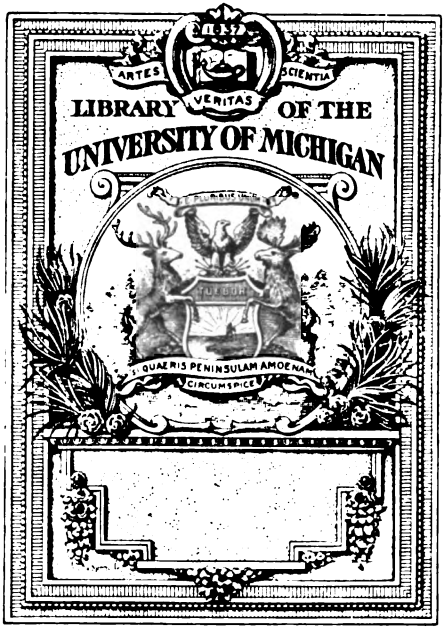

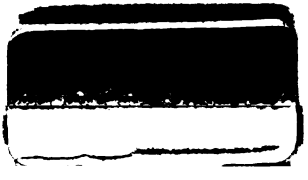

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ADDRESS

BY

CHARLES W. HARGITT,

VICE-PRESIDENT AND CHAIRMAN OF SECTION F FOR 1903.

SOME UNSOLVED PROBLEMS OF ORGANIC ADAPTATION.

With the advent of the "Origin of Species" became current the naturalistic interpretation of organic nature, epitomized in such phrases as "natural selection," "survival of the fittest," etc. So rapid and general was the acceptance of this conception as a working hypothesis that in thirty years, or within a single generation, Wallace made bold to claim for it universal recognition in the well known and oft-quoted declaration, "He (Darwin) did his work so well that descent with modification is now universally accepted as the order of nature in the organic world."

As a general statement of the fact of evolution, as the phrase may be literally interpreted, it may, after fifteen additional years of intense biological activity, be as vigorously claimed and as readily conceded. If, however, it be so interpreted as to include the full content of Darwinism and the all-sufficiency of natural selection as the prime factor, with its details of endless adaptations to environment, whether physical or physiological, it need hardly be said that consent would be far less general or prompt.

Moreover, with the highly metaphysical and speculative deductions which, under the caption of "Neo-Darwinism," or, more plainly, "Weismannism," which have boldly assumed the omnipotence and all-sufficiency of natural selection to account for the least and last detail of organic differentia-

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inseparable from the physical conditions in which they have their place.

It is different, however, with much of the color found in the organic world. While such colors as those of the grass or leaf might seem to have hardly any different explanation or to call for special explanation different from the preceding, as Wallace has pointed out, on the other hand, as he has also forcefully expressed it: "It is the wonderful individuality of the colors of animals and plants that attracts our attention —the fact that colors are localized in definite patterns, sometimes in accordance with structural characters, sometimes altogether independent of them; while often differing in most striking and fantastic manner in allied species. We are therefore compelled to look upon color not merely as a physical but also as a biological characteristic, which has been differentiated and specialized by natural selection, and must therefore find its explanation in the principle of adaptation or utility."

It is under the stimulus of this conception that the significance of color has come to have the large place and concern in the literature of evolution which it at present occupies, as expressive of which such well known phrases as "protective coloration," "warning colors," "mimicry," etc., have come to be household commonplaces among us. It is not surprising therefore that in a book like Wallace's "Darwinism" out of a total of some four hundred and seventy-five pages more than one hundred and fifty should be devoted to this phase of the problem alone, while it has frequent reference in other connections.

And the same is largely true of much of the literature dealing with the subject of organic colors. In other words, color in these relations has been studied largely, if not wholly, as a factor in adaptation—fitting the animal to better meet the exigencies of life in the struggle for existence, in certain cases serving as a disguise or screen against detection, in others by glaringly advertising some noxious quality, in still others by flying a signal of alarm or warning, and in flight serving to segregate the members of a herd in whose collective aggregate a larger measure of protection might be realized.

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Hence it naturally came to pass that color was looked upon largely as a physical factor in the sum total of the animal's morphology which must have some fundamental relation to the adaptation or fitness for survival of the species. It is not strange, under prevailing conditions, that small attention was directed to the more fundamental problem of the *physiological* significance of color, or the part it has to do in the processes of metabolism of the individual organism. Recent work in experimental morphology has directed attention to this phase of the problem, and one of the objects of the present discussion will be to make somewhat more evident a too long neglected aspect of animal biology.

It ought not to be overlooked in this connection that along with the development in experimental morphology to which reference has been made, those of organic chemistry, and particularly chemical physiology, have been perhaps equally important in directing attention to certain phases of our problem. Nor ought we to forget that the spectroscope has thrown its light upon the same general problem, though with perhaps less of conclusiveness than could have been desired. As a result of this growing activity there has been accumulated a body of information, a part of which stands directly related to the subject under consideration, and a part indirectly concerned with the same essential principles, and from it we may safely predict the solution of problems hitherto only predicated hypothetically, and such side-lights upon others equally important that it is not too much to confidently forecast substantial progress all along the line.

It may be well in this connection to glance briefly at some of the results at present known as in some measure justifying these somewhat optimistic assumptions, as well as pointing the line along which important and promising researches may be prosecuted.

The work of Krukenburg, MacMun, Macallum, M'Kendric, Hopkins, Urech, Eisig, Cunningham, and a host of others, comprising a mass of literature of enormous proportions, will be available to those interested and may afford some faint conception of the magnitude and importance of the field to

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be explored, as well as an introduction to that already made available. And while as a result of this activity many and various organic pigments have been isolated and their composition in part or entirely made known, it must be recognized that the task of the chemical analysis of any such highly complex compounds as most of these are known to be is attended with extreme difficulty and no small measure of uncertainty. Still, it has been possible to fairly distinguish several classes of such pigments, differentiated physiologically as follows:—

First.—Those directly serviceable in the vital processes of the organism. Under this head may be classed such pigments as hæmoglobin, chlorophyll, zoonerythrin, chlorocruorin, and perhaps others less known. It need not be emphasized that by far the most important of these are the two first named. The others, found chiefly among the lower invertebrates, are believed to serve a function similar to the first.

Second.—Waste products. Among these the several biliary products are too well known to call for special note. Guanin is a pigment of common occurrence in the skin of certain fishes and is associated with the coloration of the species. Similarly certain coloring matters have been found in the pigments of many lepidoptera, known as lepidotic acid, a substance closely allied to uric acid and undoubtedly of the nature of a waste product.

Third.—Reserve products. Of these there are several series, one of which, known as lipochrome pigments, is associated with the metabolism involved in the formation of fats and oils. Perhaps of similar character are such pigments as carmine, or rather cochineal, melanin, etc. It may be somewhat doubtful whether these pigments do not rather belong to the previous class, where should probably be listed such products as hæmatoxylin, indigo, etc., etc., all of which have been claimed as resultants of destructive metabolism in process of being eliminated from the physiologically active tissues of the body of the organism. Of similar character is probably tannic acid, a substance well known among plant

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products and involved in the formation of many of the brownish and rusty colors of autumn foliage, particularly of the oaks and allied trees, as are the lipochromes in the formation of the reds and yellows which form so conspicuous a feature among autumn colors.

While the association of these and other pigmentary matters has long been known in connection with both animal and plant growth, and while the conception of their more or less intimate relation to the active metabolism of the various tissues is not new, comparatively little has been done toward directly investigating and elucidating the exact nature and extent of the process. This seems to be especially the case in relation to the part played by these products in the formation of those features of coloration among organisms with which we are now concerned.

The most strenuous advocates of the primary importance of natural selection as the chief or only factor in adaptation are free to admit that among the simplest forms particularly, color has originated in some more or less obscure way through growth or some of the vital activities of the organism, Darwin, for example, merely suggesting that "Their bright tints result from the chemical nature or minute structure of their tissues," and Wallace in the even less explicit statement that "color is a normal product of organization," whatever that may imply.

So far as I am aware Eisig was among the earliest to claim that among certain annelids the colors were primarily expressions of the katabolic processes of the tissues, and were excretory in character. He was able to largely demonstrate this with species of Capitellidæ by experimental methods. By feeding the animals with carmine he was able to follow its course through the alimentary tract, its progress through the tissues, and final deposition in the hypodermal tissues beneath the cuticle, where in the process of moulting it was finally eliminated. He also found that in a species of Eunice, which fed upon sponges, the pigment granules of the food passed unchanged through the intestine and into the body tissues much as had been the case in the experiments with the preceding.

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Graff later reached very similar conclusions concerning coloration in the leeches, but was able to go a step farther than Eisig had done and to show in great detail the exact process through which it was brought about. He found in the endothelium certain migatory cells which wander about in the cœlom or penetrate through the tissues, and that among their functions one of the most important seems to be the absorption of foreign bodies and their conveyance into the mouths of the nephridia or through the tissues to the hypodermis and their lodgment in that tissue. He was even able to show that the special markings or color patterns which are so characteristic in some of the animals may be explained by the disposition of the muscle bands, and their relation to the lines of pigmentary deposition by the wandering cells, which Graff has designated "excretophores." He was also able to confirm the results of Eisig as to the experimental demonstration of feeding with various pigmentary matters, and subsequently tracing them from point to point in the process of elimination. Furthermore he showed that the amount and density of pigmentation was closely related to the intensity of metabolism, being greatest in those specimens which were most voracious feeders.

Observations of a similar character have been made upon certain of the Protozoa, particularly upon Stentor. Schuberg in 1890 found that the blue-green pigment so characteristic of this organism was constantly being excreted bodily in the form of definite granules.

In 1893 Johnson, in an extended study of the morphology of these Protozoa, confirmed the preceding observations, and showed that the pigment was excreted along with other excrementitious matter. He found also that the principal region of excretory activity was at the base of the animal, where was formed after a short time a definite mass of debris near the foot.

Perhaps one of the most important contributions along this line is that of Harmer on the character of the "brown body" of the Polyzoa. By a series of critical observations upon the life-history of these interesting organisms, and painstaking

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experiments in feeding with carmine and other pigments, he was able to prove beyond reasonable doubt that the so-called "brown body" of the Polyzoa is a direct product of the destructive metabolism within the body and is excreted in a mass at this particular region. He found that the leucocytes of the funicular organ as well as certain cells of the organ itself engulfed pigmentary wastes, and with the periodic decline of the polypides these cells became crowded into a close mass thereby constituting the "brown body." The new polypide arising by a sort of regenerative process was found to be always devoid of any coloration, no pigment appearing for some time following the activity of the new polypide, but that it is formed in regularly increasing amounts with the age and degree of metabolism of the organisms.

Correlated with these views concerning the origin of certain colors and their disposition in the organism is that of the relation of coloration to the food. It has long been known that in many cases there is a more or less intimate relation of color to the food consumed by certain animals. Instances of this are too numerous for detailed consideration here. Let it suffice that Darwin, Semper, Eimer, Koch, Beddard, Poulton, Gunther, and many others, have, by extended observations and by detailed experimentation, apparently established the general fact. Beddard quotes the following observation made by G. Brown-Goode as to such an explanation of protective coloration in fishes. "On certain ledges along the coast of New England are rocks covered by dense growths of scarlet and crimson seaweeds. The cod-fish, the cunner, the sea raven, the rock eel, and the wrymouth, which inhabit these brilliant groves, are all colored to match their surroundings; the cod, which has naturally the lighter color, being most brilliant in its scarlet hues, while others whose skins have a large and original supply of black have deeper tints or dark red and brown." He then quotes farther the suggestions of Goode that these colors are due to pigment derived either directly or indirectly from the red algæ; those which are carnivorous feeding upon the crustacea and other marine organisms whose stomachs are full of the algæ and their pigments which pass unchanged into the tissues of the fishes.

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He also quotes a similar conclusion of Gunther as to the origin of the red pigment of the salmon being derived from the red pigment of the crustacea upon which it feeds. While admitting that in the cases just cited there has been no attempt at demonstration of the proposed explanation, it yet would seem highly probable. "It is too remarkable a coincidence that the fish normally with but little pigment should when among these weeds be *bright red*, and that the fish normally possessing black pigment should be *dark red*, to permit of a settlement of the question off-hand by the easy help of natural selection—without at least some further inquiry."

With the foregoing considerations concerning the general origin and development of pigments and their relations to the colors of organisms, we may next proceed to pass rapidly in review such groups of animals as we may choose to consider, and may institute a brief inquiry as to the significance of their types of coloration as factors of adaptation.

With the avowed purpose of restricting my observations and discussion as far as practicable to the lower groups of invertebrates as already announced, it will suffice to say further that in justification of such a course I am constrained to consider the lower animals, particularly Cœlenterates, as more favorable subjects from which to obtain fundamental conclusions than are the more highly specialized insects or birds which have had so large a measure of attention in earlier investigations along these lines.

Furthermore, it seems highly probable that future investigations will involve more of direct experimentation than has hitherto been the case, and if so, these lower series will naturally afford some of the best material available for such inquiries, not only because of the more ready and rapid responses obtained, but from the relative simplicity of their organization and the consequent simplicity of results likely to be obtained in each case.

If further warrant were demanded for a comparatively limited survey, or special emphasis upon a limited group of animals, I should find it in a measure in the personal interest

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and familiarity which has come from special researches connected therewith.

Beginning with the Hydrozoa it may be noted in the outset that though including the simplest of the Cœlenterates we shall find a remarkable variety and range of coloration. Among the hydroids, as is well known, coloration is neither very remarkable as to brilliance nor distribution. Many, if not most, are almost without color distinction, except in the dull brownish or amber colors found in such as Obelia, Halecium, and other campanularians. This may be due in part to the fact that the colonies are so generally encased within a chitinous perisarc which, while somewhat colored as already indicated, is seldom if ever of any considerable brilliance or diversity. Among the Tubularians, in many of which the development of a perisarc is slight, and always lacking over the hydranth itself, there is often found considerable coloration, as in Eudendrium, Pennaria, Corymorpha, and others. And in these color is usually found associated more particularly with the development of the sexual products, or during the season of reproductive activity, which is a matter of considerable significance, to be taken up in a latter connection.

As is well known, the predominance of alternation of generations in these animals brings into prominence the sexual phase, which in most species is an independent organism—the medusa. And it is in connection with the medusæ that we find the most marked development of color. There does not, however, appear to be any well-defined distribution of colors into patterns. Among the Hydromedusæ the distribution of pigment, which is almost the only conspicuous kind of color present, is chiefly in association with the gonads, the tissues of the stomach and the regions of the chymiferous canals, though in some cases also extending to the tentacles and in the regions of the sensory organs. It should not be overlooked, however, that in many of these medusæ the color tints are among the most beautiful and delicate known, though lacking the intensity more common among the Scyphomedusæ and corals.

Turning attention to the Scyphomedusæ we find as just

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suggested a more copious development of color and also what is more significant, in many cases its distribution into something like definite patterns, as is more or less evident in such genera as Cyanea, Pelagia and Rhizostoma. It is, however, no less evident that among these we have, as in the former, the deposition of pigment along the lines of most active metabolism, such as the gastrovascular and reproductive organs, in most abundance and usually of greatest brilliance.

It is, however, when we come to the Anthozoa, which includes the corals, actinians, sea-fans, etc., that we find the climax of coloration, both as regards brilliance and intensity. To look into the crystalline depths of the waters about a coral reef where these varied forms thrive in great garden-like areas is to gaze upon a scene, the fairy-like features of which it would be difficult to exaggerate. Here are actinians, corals, sea-fans, sea-feathers, etc., etc., which abound in the richest profusion and endless variety, seeming to vie with each other in the effort to produce the most exquisite displays of every tint of the spectrum, in contributing to the splendor of the ocean garden of which they are parts.

In the distribution of color there is not apparently any advance as to differentiation over that found in the Scyphomedusæ, if indeed as much, though among the actinians certain stripings and mottlings occur over the exterior of the body. It is worthy of note that in those forms in which the tendency toward definite coloration is more evident there appears also to be in many cases considerable variation of coloration. This is particularly noticeable in such forms as Metridium and Cyanea.

Face to face with this rich profusion and beauty of color what is its significance? How has it originated and what does it mean? Is it simply the expression of some original constitution peculiar to the entire class, and if so why does it differ in so marked a degree among the different subclasses? We may safely dismiss such an alternative as altogether unnecessary and without value as an explanation. May it be considered as an adaptation to protection, the result of natural selection? Certainly in no direct sense,

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for without exception so far as I am aware the more brightly colored forms are thereby rendered correspondingly more conspicuous and therefore more liable to attack from enemies. May it come within the category of "warning" coloration, due to the offensive cnidarian armor borne by most of the members of this phylum? So not a few who have essayed an account of the matter would have us believe. It seems to me, however, open to serious doubt, aside from the fact that it lacks evidence. On the other hand among hydroids I have found that those having brighter colors are most liable to be eaten by fishes in the habit of feeding upon such a diet. Furthermore various worms, snails, etc., which are known to feed upon them would be more likely to be attracted by colors than to be repelled. It is also matter of common observation that such animals are much more abundant among colonies of highly colored hydroids like Eudendrium, Pennaria, and Tubularia than among species of Obelia or others of little color distinction. Many fishes with finely adapted dental apparatus are constant feeders upon corals, tranquilly browsing among the animated foliage of this luxuriant forest.

Finally, may it come within the category of "sexual selection"? So far as I am aware no one has ventured to assign to it any such a significance. Where sex characters are so little differentiated as among at least a portion of the phylum such an explanation would be as far-fetched as it would be unnecessary. While upon the part of some of the older naturalists there was a disposition to regard the massing of members of the Scyphomedusæ at certain times as having a sexual meaning, it may be doubted whether it has any considerable support in facts.

Concerning coloration among the Anthozoa, Duerden, whose work on the group is so extended and so favorably known, has summarized the following account:

"The prevalence of the yellow and brown color is easily understood when an examination is made of the polypal tissues. For in all instances in which it occurs, the entoderm is found to be crowded with the so-called 'yellow cells' or

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Zooxanthellæ, which are unicellular, symbiotic algæ, the chromatophores of which are yellow or yellowish green. That these are the main cause of the external coloration may be easily proved from colonies of Madrepora. In this genus the polyps toward the apex of branches are nearly colorless, and on a microscopic examination of the entodermal layer Zooxanthellæ are found to be absent while they are present in abundance in older pigmented regions."

These symbiotic algæ are not, however, the only source of color among the corals. Duerden finds ectodermal pigment granules, aggregated in somewhat irregular, isolated patches in some cases, in others somewhat regularly distributed.

He also found that a third source of coloration among corals was the presence of what he has termed "boring algæ." These were both red and green, and penetrate into the skeletal mass and color it a distinct red or green, as one or the other may be present.

In his work on the Actiniaria of Jamaica, this author has found in many cases the presence of unicellular green algæ growing upon the surface and giving to the polyp a distinctively green color. He found also superficial granular pigments in certain species which could be removed by any erosion of the ectoderm. I have found the same in several species of New England actinians, and in some cases the pigmentation was irregularly distributed, sometimes in blotches, sometimes in longutidinal stripes, more often the latter. So extremely variable is the coloration in many of these organisms that it is impossible to utilize it as a factor in differentiating species. Duerden has called attention to this feature among both corals and actinians, and believes it to be due to the presence or absence of greater or less intensity of light, and believes it to be an expression of the fact that the Zooxanthellæ are not able to thrive except under proper light, and that, moreover, where light is too intense, as in shallower waters, certain dark pigment found in such specimens is thought to be due to its utility as a screen. While there may be a measure of credibility as to phases of this view, it does not seem to me as of general adequacy. The

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variability of species to which I have just referred and to the very common genus Metridium is certainly not due in any appreciable degree to the factor of light, since it occurs indiscriminately among specimens taken in identical situations as well as under those of differing conditions.

In this connection may be mentioned the same phenomenon among medusæ. The variation of coloration in Cyanea has long been known and is so marked that the elder Agassiz distinguished two additional species chiefly on this character, both of which have long since been discarded. It is quite well known to observers that these animals when placed in aquaria usually show within a very short time a more or less marked diminution in colors. Dactylometra while living fairly well for many days in the aquarium loses within this time so much of its usually bright coloration as not to seem like the same creature. The same is true of many other animals than medusæ. On the other hand it is equally well known that many other animals may be placed under these more or less artificial environments with little apparent loss in this or other respect. That it is not due to light alone is evident in the fact that similar changes occur in medusæ which have been kept in open pools or enclosures about docks or elsewhere.

It seems to me rather that the true explanation is to be found in the changed conditions of nutrition and the consequent change in the metabolism of the animal. Hydroids placed under these conditions show the same tendency.

Those which take kindly to the change show no appreciable decline as to color or other vital process. The same is true of medusæ. Gonionemus may be kept for weeks in the aquarium, and if properly fed will show no decline in color, while if the conditions become bad an immediate change is noticeable in this as well as other features.

The same may be said concerning the actinians. While many seem to suffer noticeably when placed in aquaria others show no apparent difference. Cerianthus membranaceus, one of the finest of the actinians to be seen in the Naples aquarium, and one of the most variable, shows no apparent decline in

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any vital function. Specimens have been kept in flourishing condition in the aquarium for several years and show no sign of decline, the coloration continuing as brilliant as in the open sea. The same is true of many other organisms found in finest conditions in this celebrated aquarium. Among the annelids Protula soon shows decline in color vigor, and the same is true, though to a less degree, in the case of Spirographis and Serpula.

While it may not be without probability that some measure of this color change may be due in certain cases to the changed conditions of light, it still remains true, I believe, that light alone is but a single factor, and that often a minor one involved in the changes observed, and that changed conditions of nutrition and metabolism are by far the more important.

The main factor of our problem, however, is still unsolved. What answer shall we make to ourselves concerning the significance of the multiform colors more or less general among members of the Cœlentera? It seems to me more or less evident that natural selection can have at best but a limited place in its explanation. I see no place for it along the lines of protection, either direct or indirect.

Of even less significance can any modification of it under the guise of sexual selection be claimed; for even aside from the large majority of cases where there is slight if any sex differentiation, no sensory organization, which Darwin recognized as essential to the exercise of this factor, is present through which it might become operative in even the smallest degree.

Two, and only two, other methods of explanation have seemed to me to afford a reasonable account. First, that it is due primarily to the normal course of metabolism, during which color appears as one of its many expressions. Darwin himself was not indifferent to this possibility, and expressly states in connection with the same problem that color might very naturally arise under such conditions. "Bearing in mind," he suggests "how many substances closely analogous to organic compounds have been recently formed by chemists, and which exhibit the most splendid colors, it would have

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been a strange fact if substances similarly colored had not often originated, independently of any useful end thus gained, in the complex laboratory of the living organism." It has also been pointed out in an earlier portion of this paper that Wallace had to appeal to a similar source in his search for the primary factors of animal coloration.

Geddes and Thomson in discussing the problems of sex likewise make a similar claim. They declare, "pigments of richness and variety in related series, point to pre-eminent activity of chemical processes in the animals which possess them. Technically expressed, abundant pigments are expressions of intense metabolism." They further find in the phenomena of bright colors among the males of most of the higher animals simply the expression of the correspondingly greater activities of the processes of metabolism.

I believe that in this source we have a real account of a considerable body of color phenomena among the lower invertebrates, and particularly of that series under present consideration.

The second factor to which I would appeal is so nearly related to the former as to be involved more or less intimately therewith. It is to the effect that certain pigments are products of waste in process of elimination. This has already been referred to in a former connection and need not be separately emphasized apart from the concrete cases to which it may be applied.

Strongly significant of the importance of this process among the Hydrozoa is the fact already pointed out that pigments are found deposited along the lines of principal metabolism, namely, the gastrovascular regions, the gonads, and to a less extent the immediate regions of sensory bodies, when these may be present. While this alone as a mere statement of fact does not prove the point at issue, when taken in connection with other facts of a similar nature, it amounts to a high degree of probability.

What evidence have we that in the case of hydroids, medusæ, etc., colors are associated with excretory processes? While the facts are not numerous, they are I believe rather convincing.

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In work upon regeneration in hydroids, Driesch and Loeb called attention to certain pigmentary matters found in Tubularia and claimed for it an important function in the regenerative process. Morgan, and later Stevens, working upon the same hydroid, became convinced that the claims of the former investigators as to the importance of this pigment were not well founded. They found that not only was the pigment of no special importance, but that it was *really* a waste product, and that during the process of regeneration was actually excreted and finally ejected bodily from the hydranth. I have personally been able to confirm these results on the same and related hydroids, and have also shown that in regenerating medusæ there is formed *de novo* in each regenerating organ, such as manubrium, radial canals, etc., the characteristic pigment of the normal organ. This was particularly noticeable in the case of radial canals. Following their regeneration and promptly upon their functional activity the deposition of pigment made its appearance, and within a comparatively short time had acquired the normal intensity. This was also true of other organs, tentacles and tentacular bulbs, as well as manubrium and canals.

Substantially the same results have been obtained, though here first announced, in experiments upon one of the Scyphomedusæ. In very young specimens where the tissues are delicate it is possible to note the intense activity in regenerating organs, such as sensory body. The first part of this organ to make its appearance is the sensory papilla, which is soon followed by the otoliths, and later by the special pigmentation of the entire organ.

From the foregoing considerations three things seem to me to be more or less evident:

First.—That in all regenerative processes a very marked degree of metabolism is involved, whether in the mere metamorphosis of old tissues into new, or in the direct regeneration of new tissues by growth processes, both of which seem to occur.

Second.—That in regenerative processes there is often associated the development of pigmentary substances which seem to have no direct function in relation thereto.

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Third.—That in many cases there follows a more or less active excretion and elimination of portions of the pigment in question.

Concerning color phenomena among the several classes of worms we are in much the same uncertain state of mind as in the former. For while in some of the annelids there may be found fairly well developed visual organs it may be seriously questioned whether they are of any such degree of perfection as would enable their possessors to distinguish small color distinctions. And if this be the case there would at once be eliminated any possibility of conscious adaptation in seeking a suitable environment, or such as would be involved in so-called sexual selection.

Furthermore, it is very well known that among this group some which exhibit among the richest of these color phenomena have their habitat in seclusion, buried in sand or mud, or hidden beneath stones, or with tubes built up from their own secretions, or otherwise so environed as to render practically nil the operation of natural selection.

Again, it should not be overlooked in this connection that in many of the annelids, as well as others, the most pronounced source of color is to be found in the hæmoglobin dissolved in the blood, and that it would be as futile to ascribe its color to natural selection as it would to claim a similar explanation of the color of the same substance in the blood of vertebrates, where as color it is absolutely of no selective value, except in such special cases as the colors of the cock's comb, where it may come to play a secondary function as a sex character.

What shall be said of such forms as Bipalium and Geoplana among land planarians, which exhibit in many cases brilliant coloration, but since they are chiefly nocturnal in their habit and conceal themselves during the day under logs or other cover, the color could hardly serve any selective or adaptive function?

The same is equally true of such forms as Nemerteans whose habitat is beneath the sand along the tide line or below, and also of many annelids having a similar habitat. Some of these, particularly among the latter, have types of coloration

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which are often of brilliant character and splendid patterns, vying, as one writer has expressed it, "with the very butterflies."

It cannot be questioned that in some cases we find among these forms what would seem at first sight to be splendid illustrations of protective coloration. If, however, we trace in detail their distribution and variable habitat we shall often find, as did Semper in the case of Myxicola, that the supposed case of marvelous mimicry resolves itself into merest coincidence. This case cited by Semper is described in detail in "Animal Life," and its careful study by some of our over-optimistic selectionists would prove a healthy exercise, conducing to a more critical scientific spirit and, as a consequence, to saner interpretations of appearances in the light of *all* the facts.

The mimicry in the case was of coral polyps among which the annelid was found growing and which, in the form of its branches, their size and coloration, seemed so perfect that it had long escaped notice and was described by Semper as a new species.

It was found in various localities among the corals, but invariably having precisely the same simulation of the polyps, so that Semper noted it as among the finest cases of mimicry which had come to his attention. It so happened, however, that soon after he happened to discover his mimetic Myxicola growing upon a sponge whose color and form were so different as to render it very conspicuous. A systematic search for it in other situations soon revealed it among the rocks, and in his own language, "Almost everywhere, and wherever I examined it carefully, it was exactly of the size and color of the polyps of Cladocora cæspitosa."

Attention has already been called to Eisig's account of coloration among the Capitellidæ, in which he discards the factor of natural selection as wholly inadequate in the case of the organisms under consideration as well as in many others, and refers to many investigators who have likewise found it deficient. In his exhaustive monograph the subject is discussed in considerable detail and references given which it would be impracticable to cite in such a review as the present.

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It will be possible to refer but briefly to another group or two in the present discussion, the first of which is the Echinoderms, and chiefly the starfishes. As is well known these organisms exhibit a considerable range of variety and richness of coloration, among which red, orange, brown, yellow and black are more or less common. In not a few cases of course the colors comprise combinations of two or more of those named. An examination has been made of these pigments in a few cases and has sufficed to show that for the most part they are lipochromes, and therefore belong to either reserve or waste products. Similar colors are also found among the brittle-stars, with occasional admixtures of blue or green, colors less common in the former group.

As is also well known similar colors are found among the Crustacea, into a consideration of which it is impossible to enter here. There is a matter, however, which I cannot ignore in connection with the group, namely, the rather remarkable fact that in two phyla having so little in common as to habit, structure or environment, there should be so striking a color resemblance. This is further heightened by the fact that while the one is a prey to almost every denizen of the sea of predatory habit, the other is almost correspondingly exempt. So far as I know Echinoderms have few enemies, and are of course largely invulnerable against such as might otherwise find palatable feeding among these sluggish herds. If the color is in the one case protective, why not in the other? Or if it be not protective on the other hand, why claim such in the first? That sexual selection might have some place among Crustacea may not seem improbable. But if color is its signal here what does it imply among Echinoderms, where in the nature of the case it must be ruled out of account?

Discussing the significance of colors among the Echinoderms Mosely submits the following interesting problem: "Those coloring matters which, like those at present under consideration, absorb certain isolated areas of the visible spectrum, must be considered as more complex, *as pigments*, than those

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which merely absorb more or less of the ends of the spectrum. . . . It seems improbable that the eyes of other animals are more perfect as spectroscopes than our own, and hence we are at a loss for an explanation on grounds of direct benefit to the species of the existence of the peculiar complex pigments in it. That the majority of species of Antedon should have vivid coloring matters of a simple character, and that few or only one should be dyed by a very complex one, is a remarkable fact, and it seems only possible to say in regard to such facts that the formation of the particular pigment in the animal is accidental, *i. e.*, no more to be explained than such facts as that sulphate of copper is blue."

Considered from the standpoint of metabolism such facts would hardly seem to assume the difficulty which might be implied in the case just cited, indeed they are in perfect alignment with what might be anticipated, and what has in cases previously cited been found to be actually occurring.

Similar conditions as to color and color significance are also matters of common knowledge in relation to Mollusca. Perhaps few groups among animals exhibit more brilliant and varied colors than are to be found among Gasteropods, yet in many of them this factor can have no more value as a means of adaptation than do biliary pigments or Hæmoglobin among vertebrates, where as pigments their significance is nil. Of them, Darwin with his usual frankness, has said, as previously cited, "These colors do not appear to be of any use as a protection; they are probably the direct result, as in the lowest classes, of the nature of the tissues—the patterns and the sculpture of the shell depending on its manner of growth." Referring in the same connection to the bright and varied colors of Nudibranchs, he further declares, "many brightly colored, white, or otherwise conspicuous species, do not seek concealment; whilst again some equally conspicuous species, as well as other dull colored kinds, live under stones and in dark recesses. So that with these nudibranch molluscs, color apparently does not stand in any close relation to the nature of the place which they inhabit."

Into the classic shades afforded by the insecta as a fruitful

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haunt and stronghold of natural selection I must not venture. Not that its problems have all been solved, nor that some considered as settled beyond controversy may not have to be readjusted, not excepting the much exploited Kalima itself, but out of pure regard for the exigencies of the occasion.

No more dare I presume to enter the abysses of the deep sea and to pass in review its manifold and almost untouched problems of color significance, great as is the temptation and attractive as are its inducements. It must suffice to suggest that had half the ingenuity which has been exercised to bring these problems into alignment with the general sway and supposed supremacy of natural selection been employed in an analysis of the pigments and some efforts made to discover the origin of coloration and its general significance as a physiological, rather than as a physical one, we should have been saved the sad rites attending the obsequies of still-born hypotheses and half developed theories. The desperate attempt to save natural selection from drowning in its submarine adventures by lighting its abyssal path with the flickering and fitful shimmer of phosphoresence was worthy of a better cause. It is difficult to be serious with this phase of a subject the nature of which demands anything but ridicule or satire. But the attempts to illuminate the quiescent abysses with the dull glow which under all known conditions requires, if not violent, at least vigorous stimulus to incite it, and the assumption that its sources were sufficient to meet even a moiety of the necessities involved, makes a draft upon one's credulity which might arouse either indignation or the sense of the ludicrous, depending upon the point of view! But seriously, such a conception apparently loses sight of too many evident known conditions of phosphoresence with which we are familiar, not to mention the growing belief that the phenomenon is in itself of the nature of one of the wastes of metabolism, to justify the herculean attempt to make it serve a cause so desperate.

As a concluding word allow me to say that in the present review I have not in the least sought to ignore or discredit the value of natural selection as a factor in organic evolution.

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Nor would I be understood as wholly discarding color as a factor in organic adaptation, particularly among the higher and more specialized forms. At the same time I must submit to a growing conviction that its importance has been largely overestimated, and that other factors have been as largely lost sight of. If the present discussion may serve in even the smallest degree to direct attention to some of the latter it will have served its chief purpose.

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NATURAL HISTORY OF HAMINEA SOLITARIA SAY.

W. M. SMALLWOOD.

THE shell-bearing species of the family Bullidæ have been found freely distributed in the rocks since the Tertiary period. A large number of species have been found as fossils. Ludwig in Leunis *Synopsis der Thierkunde* states that there are between two and three hundred recent species. They have a wide distribution in both European and American waters, being commonly found in the sandy and muddy bays of the temperate regions.

The Bullidæ belong to the general order of Opisthobranchia and to the suborder Tectibranchia.

Thomas Say read before the Academy of Natural Sciences at Philadelphia on July 24th, 1821, a paper in which he gives "An account of some of the marine shells of the United States." In this article we have the first reference to *Bulla solitaria*. His description is as follows: "Bulla solitaria. Shell remarkably thin and fragile, pellucid, oval, narrowed at base, with numerous impressed, revolving lines, and transverse very obtuse wrinkles; apertures surpassing the tip of the shell; spire none, substituted by an umbilicus; umbilicus of the base none, less than half an inch. Inhabits the southern coast of the United States.

July 4th, 1835, J. G. Totten described a species of Bulla, variety *insculpta* which he dredged in about fifteen feet of water from the muddy bottom of the harbor at Newport, R. I. He maintains that *insculpta* is distinct from *solitaria* because of some slight variations in the color and shape of the shell. He says, "Thus (insculpta) can hardly be Say's Bulla solitaria. It is not umbilicated at the top as that species is; having merely a shallow pit in which nothing of the interior whirls can be seen. The solitaria is described as being narrowed at the base; but though our shell is regularly rounded in the passage, below, of

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the right into the left margin, it is widely rounded; and the widest part of the shell is below the middle."

A. A. Gould, in his *Invertebrata of Massachusetts* ('41), considers *Bulla insculpta* and *Bulla solitaria* identical: he concludes his description of *Bulla insculpta* with the following paragraph: — "The differences between solitaria and insculpta, if there be any, must be very slight. Nor do I see that the two descriptions (Totten and Say) are at all inconsistent with each other. Still it is true that the shells from Martha's Vineyard are precisely like those from Charleston, S. C., and accord with Mr. Say's solitaria; and those from Roxbury are precisely like those found by Col. Totten at Newport, R. I., and described by him. The observable differences are, that the first are of a more dead white, are more cylindrical, the summit has a more square appearance, the revolving lines are less distinct, and there is always a perceptible opening in the region of the spire. These differences may be ascribed to age or locality."

Verrill ('73) describes *solitaria* as occurring along the Atlantic coast from Massachusetts Bay to South Carolina and makes no mention of *insculpta* except to assume that it is the same species as *solitaria*. The shells of *insculpta* and *solitaria* as figured by Totten, Gould, and Verrill seem to be identical.

The size of the shells which I have found at Wood's Holl, Mass., varies to a considerable degree; one shell found was only one-fourth as large as the average, the shape of a large number of shells which were examined varies in regard to the width and length of the lip. All of the shells examined were dextral. The distinctness of the revolving lines seems to depend on the age, the larger ones being the more distinctly marked. The statement that the shells of *insculpta* seem to be more of a dead white color than that of *solitaria* is easily explained. The shell of the living animal is a golden gray; the golden tinge being given by the thin structureless membrane, the periostracum, which completely covers the shell (Fig. 1). When this membrane has suffered disinte-

FIG. 1.— *Haminea solitaria* showing the proboscidial snout and tentacular disks. The parapodial folds conceal the anterior part of the shell and the mantle extends posterior to it. Natural size.

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gration the shell has the dead white color mentioned by Gould. In view of these variations I think that the shell as originally described by Say is identical with the *insculpta* of Totten and is the *solitaria* found in the vicinity of Woods Holl, Mass., and of Long Island, N. Y.

Pilsbry has decided to limit the family Bullidæ to the single genus Bulla. Formerly the members of the Akeridæ were included with the Bullidæ but the character of the radula, form of the shell, and relation of the animal to it enables one to sharply differentiate the two families.

The Bullidæ have in the radula a few longitudinal rows of teeth (formula I. 2. I. 2. I.). The animal is capable of complete retraction into the shell. There are no epipodal or parapodal lobes; the foot is long and tapering behind. The shell has a mottled color pattern.

The animal described by Say, Totten and others and referred to in this article is placed by Pilsbry[1] in the family Akeridæ and in the genus Haminea. "The Akeridæ are a much lower stock of Tectibranchs than Bullidæ, retaining the primitive multi-dentate radula, and the epipodal lobes, and having a thin, fragile unicolored shell.

The following is the specific description of *Haminea solitaria*: "Shell thin, subcylindrical, with gently convex sides, truncate vertex and rounded base; color horny or light brown. Surface shining, having irregular growth wrinkles and (under a lens) *fine, deeply impressed spiral grooves*, much narrower than their intervals, sometimes with smaller ones intercalated. Vertex white, somewhat impressed in the middle, subperforate. Lip arising to the right of the center, slightly thickened; outer lip gently arched forward. Columella thin, concave. Alt. 10, diam. 6½ mill" (Fig. 2).

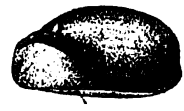

FIG. 2.—The shell of *H. solitaria*. × 2.

Habitat.—Until the report of Verrill, ('71–'72) nothing was said of the animal or its natural history. All writers confined themselves to a conchological description of the species. Verrill says that *solitaria*

[1] *Manual of Conchology.*

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"is restricted to muddy shores and bottoms, in sheltered situations and is found also in muddy ponds and estuaries." Smith and Prime state that the animal is rare, found in mud at a depth of one or two fathoms. Balch ('99) states that the animal, "Haminea solitaria, Bulla solitaria, is rather uncommon alive, sometimes occurs on marsh grass, top of sea walls." In regard to the general distribution of this species Verrill says, "From Mass. Bay to South Carolina it is common in muddy lagoons and salt ponds, in shallow water where not too brackish, along the shores of Vineyard Sound, Buzzard's Bay, and Long Island Sound. Abundant in a small pond near Holme's Hole, in New Haven Harbor, in ditches near Fort Hale."

Haminea solitaria is found rather commonly in the Eel Pond and Big Harbor at Wood's Holl, at Hadley Harbor, and at the bathing beach on Buzzard's Bay at Wood's Holl. During the laying season the animal migrates into shallow water and may occasionally be seen upon the eel grass and algæ, but usually not in water less than two feet deep at low tide. If the eel grass be disturbed they drop at once into the water and fall to the bottom, where it is very difficult to see them, owing to their form and color being so similar to the ordinary weeds and mud at the bottom; thus it is practically impossible to gather animals by looking for them on the weeds or bottom. They have been secured in two ways: first, by taking an ordinary fine-mesh dip net and skimming off the sea weeds and half an inch of mud from the bottom, then carefully washing out the mud; in this way a number have been secured in the shallow ponds and lagoons about Wood's Holl; secondly, by dredging; this method is necessary in deep water. Bulla may be found, even in the breeding season, in water thirty feet deep in the Big Harbor at Wood's Holl, — the greatest depth where they have been found. This peculiarity may be due to the fact that their former laying spot in shallow water is now occupied by a deep sea wall. Dr. Charles B. Wilson obtained a number of *solitaria* by skimming in clear water near Edgartown about the 18th of August. He was sure that the net did not pass over any eel grass or sink beneath the surface. I feel sure that this is the real explanation of the occurrence of Bulla in great abundance

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just before the egg-laying time, and their equally sudden disappearance after the egg-laying time. They simply rise to the surface and swim away.

In the summers of 1897 and 1898 most of the specimens obtained by me were collected in the larger of the two so-called "oyster ponds" near the Marine Biological Laboratory. Although a large number of capsules were taken from this place, over one hundred capsules were gathered from another locality and put into this lagoon in order to assure an abundance of material in succeeding seasons. In the summer of 1899 no capsules could be found in this lagoon, in the summer of 1900 three capsules were found here, but all of the eggs had died before reaching the twenty-cell stage; the tide flows freely into the lagoon thus keeping the water comparatively fresh. The drainage from the Marine Biological Laboratory flows into an adjacent lagoon and it is possible that the water seeping through may have some poisonous effect which kills the eggs and has destroyed or driven out the animals themselves.

The Bulla found on the sandy bathing beach at Buzzard's Bay occurs about half an inch below the surface of the sand; usually near the capsule, which is the only indication of the presence of the animal.

General Morphology.[1] — It is not my purpose in this section to go into the details of the various systems of organs except in so far as they are directly related to the development of the egg; I purpose, however, to give a brief sketch of the general form of the animal.

Probably the most striking feature of this species is its color, which is a golden gray thickly mottled with dark brown and occasional orange spots; one might almost say that the gray background looks as though sprinkled with fine sand. The shell is translucent and slightly striated spirally; it is not at all glossy or shiny. One would hardly expect to find much of a shell in a tectibranch, but in *solitaria* the reduction of the shell

[1] For a full discussion of the Morphology of the family Haminea (Bullacea) see M. Vayssiere, Recherches Anatomique sur Les Mollusques de La Familles des Bullides *Ann. Sci. Nat.* Tome 9. 1879. R. Bergh, Bullacea. *Reisen in Archipel d. Philippinen*, pp. 211–312, 1901.

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has only just begun. The most noticeable feature in this process is the fact that the shell is thin and fragile, so much so that it must be handled very carefully. The second feature is that in the mature animal the shell is not large enough to shelter the whole body in its contracted state. The shell certainly cannot afford particular protection against enemies, but when we remember where the animal lives and its habit of crawling along the mud and sand, we can readily see that it could have no better protection than its color. It imitates the color of the roots of eel grass most closely; at first sight I have often mistaken the detached fragments of these roots for Haminea.

The size of the animal depends largely upon age; the smallest shell measured was three mm. in width, and five mm. in length; the average size of the mature shell is 7 mm. in width, and 10 mm. in length. The average length of the extended animal including the shell is 2 cm.; the smallest animal was 8 mm. in length. I can make no definite statement as to the age or size at which *H. solitaria* begins to lay, but can say that I have never known the smaller ones to lay. In the aquaria the animals die after laying, but I do not believe that this fact can be used as proof that they always do.

The foot of Haminea possesses, though not clearly distinguished, the three divisions characteristic of the typical molluscan foot. From the mesopodium arise the parapodia; these are lateral lobes, or folds, like extrusions of the edges of the foot. In some of the Opisthobranchia the parapodia (epipodia of Pilsbry) are highly developed and unite upon the back, completely covering the shell. In our species however the parapodia cover only the anterior part of the shell.

The young animal moves by stretching forward the head and foot, and then drawing the visceral mass forward, so that the progress results from an alternate stretching and contraction of the anterior flexible part of the body. The posterior part being drawn ahead at each contraction. In the mature animals there is no such noticeable division in the movement; with them locomotion is apparently a continuous gliding process.

The anterior part of the animal terminates in a broad thin proboscidial snout. Running back from the end of the snout

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there are two tentacular disks, which are divided only part way. There is very little movement in these disks, except as they shift about over the anterior portion of the shell. In this species there seem to be no special sense organs in the tentacular disks. So far, I have been unable to explain their function. The tactile sense is usually located in the tentacles, but in our animal the anterior portion of the snout performs this function, as is very evident from its movements. In many of the Opisthobranchia the mantle folds over part of the shell, but in *H. solitaria* the only external evidence of the mantle is a thick fold which occupies the lip of the shell and extends back of it for about four mm., it is here extended and affords a surface equal in width to the shell. At first sight one would think because of this arrangement that the foot extended from the snout to the posterior portion of the animal, but closer inspection shows a sharp demarcation which indicates the boundary between the posterior portion of the meso-podium and the mantle. All of the parts of the body that are exposed are covered with columnar, ciliated epithelium. A great quantity of mucus is secreted by the animal, so that in crawling about the dish they often leave a nearly perfect tube of mucus. This is secreted by numerous typical single-celled glands, which are especially abundant at the edges and tip of the snout, and the outer portion of the tentacular disks.

The especial characters aside from the shell which distinguish *H. solitaria* are the stomach plates and the form of the radula.

FIG. 3. — Showing the arrangement of the stomach plates, each of which is imbedded in a large amount of muscle. The free surfaces are so arranged as to triturate perfectly the food.

There are three stomach plates so arranged that the food is trituated by them (Fig. 3). The plates are composed of chitin, having their bases firmly imbeded in the strong muscles of the stomach. The form of a single plate is shown by the camera drawings (Figs. 4, 5). The portion of the plate that comes in contact with the food is differentiated into a number of ridges which are larger and more pronounced at the anterior end. The

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largest teeth are found on the anterior part of the plate, having a triangular form. The size and prominence of the teeth gradually decreases toward the posterior part of the plate. This gradual increase in size of the teeth and the ridges indicates how both are continually forming to take the place of the more anterior ones as they become worn out. The youngest teeth are those found on the smallest ridges. In some instances it is difficult to be certain that teeth are even present.

FIG. 4 — One of the chitinous stomach plates as seen from the free surface. × 24.

FIG. 5. — One of the chitinous stomach plates as it appears from a profile view. × 24.

The radula is an interesting structure but one that is difficult to represent in a drawing. When this organ is removed from the animal, it is somewhat triangular in shape (Fig. 6). In the center of the anterior part there is a single row of teeth, having a broad free margin and terminating at each side in a rather broad blunt process (Fig. 7). There are an indefinite number of long, slender, sharp teeth arranged in rows which correspond to the teeth of the median ridge. The bases of these lateral teeth are imbeded in the muscles of the radula (Fig. 8). Each lateral tooth is bent at an angle of about 90 degrees. This arrangement of the teeth would give the following formula for *H. solitaria* ∞. I ∞.

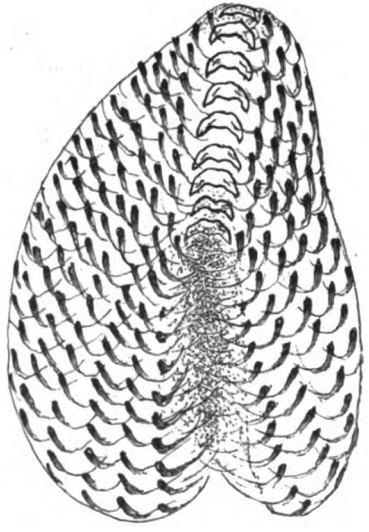

FIG. 6. — The radula seen as a transparent object. The anterior end is uppermost in the drawing. The formula is infinity. one. infinity. × 72.

The genital organs.— This system of organs is fully discussed by Lang; I have been able to demonstrate of all the

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parts as given by him and will, therefore, quote freely from his description. The Opisthobranchia are all hermaphrodites. Haminea is no exception to the general rule and comes under the first type as described by Lang. The germinal gland consists of numerous "converging diverticula." The eggs and spermatozoa arise from the same part of the gland and are intermingled. During their development they become detached and lie free in the cavity of the gland. The ducts of *H. solitaria* are considerably complicated, because of the development of the accessory organs. The ovotestis lies between the lobes of the liver and the posterior part of the body; this gland has numerous branches, which finally collect into the common hermaphroditic duct; the duct empties into the common genital cloaca. The genital aperture opens into the extreme right anterior part of the mantle cavity, and from it there is, continued forward, an open ciliated furrow, which carries the spermatozoa to a gland called the "prostate"; this opens into the penis. "The penis itself lies in the right, on the boundary between the head and foot. When it is at rest its sheath lies in the cephalic cavity near the buccal mass." Two important glands open into the genital cloaca by a common duct; first, the albumen gland, which is comparatively small and lies upon the surface of a second, known as the nidamental gland; the latter is much larger than the former and yields the outer protective envelope of the egg. The albumen gland supplies the albumen for the egg capsule.

FIG. 7.—Three of the median plates from the radula. × 390.

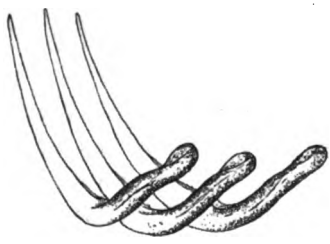

FIG. 8.—Three of the lateral teeth from the radula. × 390.

The "receptaculum seminis" is connected by a short duct with the genital cloaca and receives the spermatozoa at the time of copulation. Of course the size of this vesicle varies according to the season and according to whether or not it is full of spermatozoa.

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Breeding habits.— In general the breeding season of our species extends from the last of June to the first of September. There seems to be considerable variation in the time when the egg laying begins, in 1897 the capsules were first found July 9th. In 1898 none were found until July 25th. Then three were found which had been laid within thirty-six hours. In 1900 collecting was begun July 10th and capsules were found which must have been layed as early as June 25th, judging from the fact that the embryos had already left some of the capsules and were leaving others. However, the animals lay most abundantly between July 15th and August 15th. The egg capsules may be found scattered through the eel grass and algæ on the edges of the pond, or lagoon, but each capsule is attached to grass or algæ from two to six feet below the surface of the water. Apparently the animals congregate in favorable spots to lay their eggs. In one place in the eel pond, where the water is about three feet deep at low tide, over five hundred capsules were collected in ten days within an area about ten feet square. During the same period repeated trips were made to various parts of the pond, but only a very few capsules were found.

On the sandy bathing beach previously mentioned the animals lay in the same place from year to year. Here there is a small patch of eel grass in about three feet of water; in and around this patch the eggs are laid in great numbers. In this locality the capsules are attached either to the eel grass or, as in most cases, simply to the sand. The sandy bottom slopes out gradually for about one-fourth of a mile, merging finally into a muddy bottom. I have never dredged here, either before or after the breeding season, so I do not know where Haminea stays during other seasons; but I have dug down into the sand, both before and after the laying season, and have never been able to find any of the animals. It is difficult to ascertain where Haminea lives at other times than the breeding season, but the fact that it cannot be found in shallow water except at this time seems to show that it migrates into deeper water. This idea is also supported by the occurrence of Haminea in thirty feet of water in the Big Harbor, and by the statement of Verrill ('72) to the effect that, "A specimen of winter flounder

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(*Pseudopleuronactes americanus*) caught at Wood's Holl in August, contained a large number of the shells of Bulla solitaria." The winter flounder is dredged in the vicinity of Wood's Holl at a depth of about fifteen to thirty feet, and is mentioned by Verrill as devouring Bulla in large numbers. Haminea has been dredged in May in Hadley Harbor at a depth of twenty-five feet. These facts would tend to show that its natural habitat for the most of the year is the deeper water. A further proof that *H. solitaria* lives in deep water except during the breeding season is supplied by the following facts: August 28, 1900, Dr. C. V. Wilson while skimming the surface water with a fine mesh net near Edgartown, secured a large number of *H. solitaria*. They all died during the first night although they were placed in a small aquarium. The time that these individuals were collected was after the usual egg laying periods for the forms that live in the vicinity of Wood's Holl. When taken, they were evidently migrating into the deep water. The observations of Verrill and those of Wilson seem to show conclusively that *H. solitaria* is a deep sea dweller except for about six weeks, when it takes up temporary quarters in some favorable place in shallow water.

During the first two summers that *H. solitaria* was under my observation I was unable to discover any evidences of copulation, owing to the fact that early in the season I did not have in the laboratory any considerable number of animals; but in the summer of 1900 I had in the laboratory about forty animals at one time and was able to make observations on copulation. The habit which these molluscs have of crawling over one another and of collecting into a pile, concealed the fact and method of copulation for a long time. One day, however, I noticed two animals apparently copulating, the genital grooves were slightly extended and came together, from one the penis protruded into the genital groove of the other. The animal receiving the penis laid a mass of eggs eight hours after the copulation, the other one did not lay. In a second case of copulation the animal laid after the lapse of thirty-six hours, but this was an unusually long time and probably due to the unnatural conditions surrounding it. In the time that elapses between the period of copulation and deposition, *H. solitaria* is similar to many of the nudibranchs (Smallwood :03).

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The question arose as to whether or not copulation took place after laying; in the three cases that I was able to observe copulation did take place after one of the animals had laid but I was unable to observe it between animals both of whom had already laid. Fresh Haminea were collected, put into separate dishes, and kept there for three days; during this period they did not lay; then specimens which had copulated and laid were put in with these, copulation followed in each case in about an hour and the animals laid in about twelve hours. Sections of the ovotestis before and after copulation prove the correctness of the above observations.

In copulation the animals do not uniformly assume any definite position in regard to each other. The genital groove opens on the right side just anterior to the lateral fold of the parapodium, thus obviating the necessity for an exact position. Copulation continues for about fifteen minutes.

This species lays a single gelatinous mass (Fig. 9) which is spherical, about three-quarters of an inch in diameter. Its contents are chiefly composed of albumen, which is secreted by the albumen gland. As soon as the albumen comes in contact with the water it swells by the rapid absorption of water, and thus affords a gelatinous protection for the egg. When the eggs first leave the genital groove they are in strings; in a few hours the strings lose their continuity and the eggs are scattered throughout the egg mass. It would be very difficult to count the eggs in a single mass. The size of the capsule varies considerably; as a rule those found on the eel-grass are about a third less in diameter than those laid on the bottom. The egg masses laid in the laboratory were often irregular in shape and much smaller than those collected from the pond. The specimens in confinement that laid small and irregular masses, often laid a second time without a second copulation. It takes from 40 to 50 minutes for an animal to lay a complete normal egg mass.

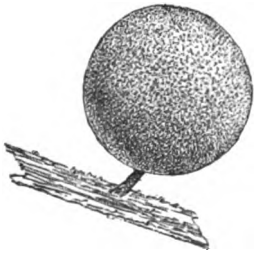

FIG. 9.—The eggs of *H. solitaria* are laid in a gelatinous mass, spherical in form, and attached to some foreign object. The drawing is natural size.

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The living egg of *H. solitaria* is so small and so richly supplied with deutoplasm that satisfactory observations on the segmentation are impossible except in the early stages. The egg is spherical, enclosed within a thin structureless membrane. The size of the egg varies, the average is about .08 mm., being smaller than the eggs of Umbrella (Heymons, 93), Crepidula (Conklin, '97), Nucula (Drew, :01) and that of most molluscs that have been studied.

Before segmentation the polar differentiation of the egg is but slightly indicated, the yolk being almost uniformly distributed except in the region of the polar bodies. It has already been

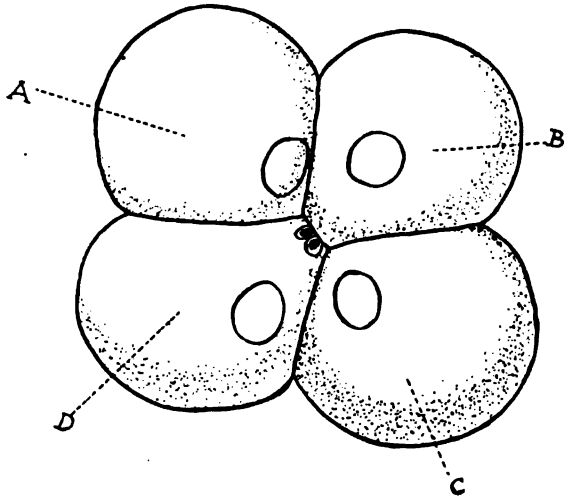

FIG. 10.— The four celled stage which shows the relation between the four blastomeres characteristic of Mollusca. × 275.

stated that it takes forty minutes for the animal to lay a mass of eggs. Within ten or fifteen minutes after each egg is laid the first polar body appears at the animal pole and thirty minutes later the second polar body can be seen. It happens occasionally that the first polar body is very large and may even contain yolk spheres.

The egg segments into two cells a half hour after the second polar body has appeared. In about thirty per cent. of the eggs observed, the first division of the egg did not divide it into two equal blastomeres, one being noticeably larger, a variation which

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is similar to Umbrella (Heymons '93). Within thirty or forty minutes after the formation of the two celled stage, the four

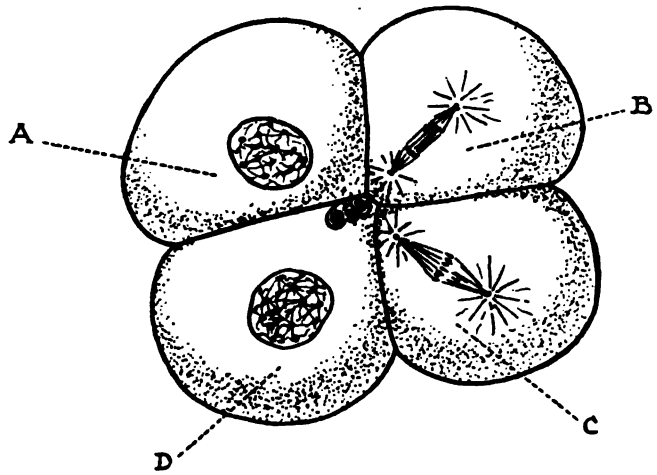

FIG. 11.— The four-celled stage preparatory to the formation of the first quartette of micromeres. × 275.

celled stage is formed. Just prior to the formation of the four celled stage, the spindles do not lie parallel which is an agreement with other molluscs and indicates a spiral division [1] (Fig. 10).

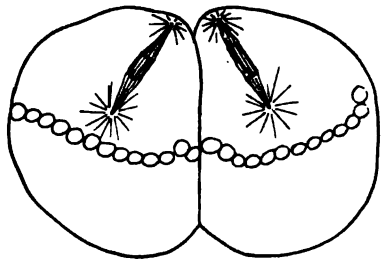

FIG. 12.— A section of the four celled stage passing into the eight celled stage to illustrate the position of the spindles when the micromeres are formed. × 140.

After not more than thirty minutes, the third cleavage separates the egg into two conspicuous parts, the protoplasmic micromeres and the deutoplasmic macromeres. These micromeres are considerably larger in comparison with the size of the macromeres than in many molluscs. The same is true for the two following quartettes of micromeres

[1] For a complete discussion of the method and significance of segmentation in Mollusca see Mark, E. L., Maturation, Fecundation, and Segmentation of Limax campestris. *Bull. Mus. Comp. Zool.* vol. 7. 1881. Conklin. E. G., The Embryology of Crepidula. *Jour. Morph.* vol. 13, 1897. Holmes, S. J., The early development of Planorbis, *Jour. Morph.* vol. 16, 1900.

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which results in the complete envelopment of the macromeres at an early stage. The third cleavage is dexiotropic (Figs. 11, 12, 13, 14).

The time that intervenes between the formation of the second and third quartettes of micromeres is the same as that for the second and third cleavage. From this time on it was impracticable to follow the further cleavage stages on the living egg. Stained preparations confirmed the observations made on the living egg.

The second quartette of micromeres is formed by the fourth cleavage which takes place in an anti-clockwise direction, the

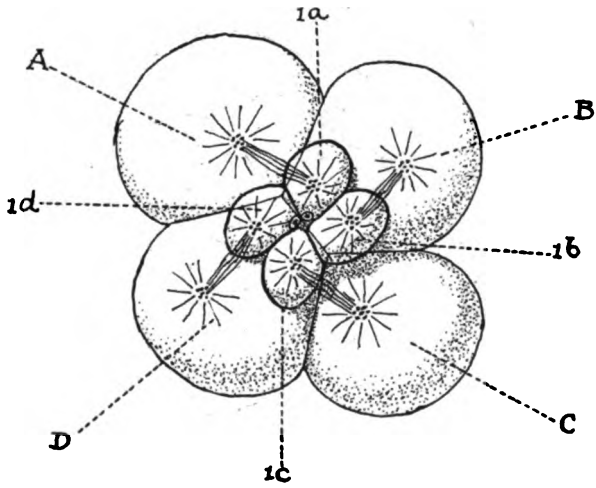

FIG. 13.—The telophase in the formation of the first quartette of micromeres. The movement is dexiotropic.

cells taking a position alternating with the cells of the first quartette (Fig. 15). The next cells to undergo segmentation are the first micromeres formed, segmentation takes place in a læotropic direction giving rise to the turret (trochoblast) cells (Figs. 15, 16); immediately after, indications of division in the second quartette of micromeres and the macromeres are evident. The egg sketched shows eight complete spindles in the metaphase. The spindles occurring in the macromeres participate in the formation of the last quartette of micromeres, the movement being in a right handed spiral. Soon after the above described

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changes have taken place the macromere which is designated by the letter D divides independently of the three other macromeres into two cells of unequal size but both containing yolk spheres. The smaller cell is concerned in giving rise to the mesoblastic bands and is entirely covered above by the micromeres.

It can be seen from the brief description of the early segmentation stages and the accompanying sketches that *Haminea solitaria* does not exhibit any segmentation phenomena other than those characteristic of mollusca. The subsequent cleavage

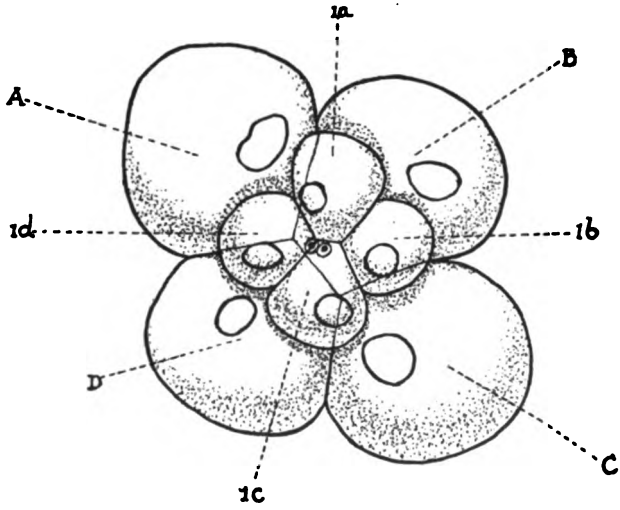

FIG. 14.—The eight celled stage fully formed showing the position of the micromeres above the furrows of the macromeres. × 275.

stages have been followed sufficiently to indicate that they are in agreement with related forms that have been described heretofore.

The embryo usually begins to move in the egg capsule at the end of the seventh day; the cilia on the mantle border are well differentiated and can be seen in motion. It is difficult to ascertain the exact length of time the embryo remains in the egg capsule but I have known it to continue there for a week. In most of the egg masses that have been under observation the embryos have died before becoming free swimming individuals although some were immediately placed in aquaria or in the

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eel pond in bottles closed with several thicknesses of cheese cloth. In placing the embryos in their natural environment, it was hoped that it would be possible to determine the changes taking place between the embryo and the adult. Although repeated experiments were made in various parts of the eel pond, no satisfactory results were obtained and I am unable to state how the transition from the embryo to the adult takes place.

During the summer of 1899 a number of pressure experiments were tried for the purpose of determining the effect on segmentation and the subsequent history of the embryo. The fact that the eggs are surrounded by a great mass of albuminous

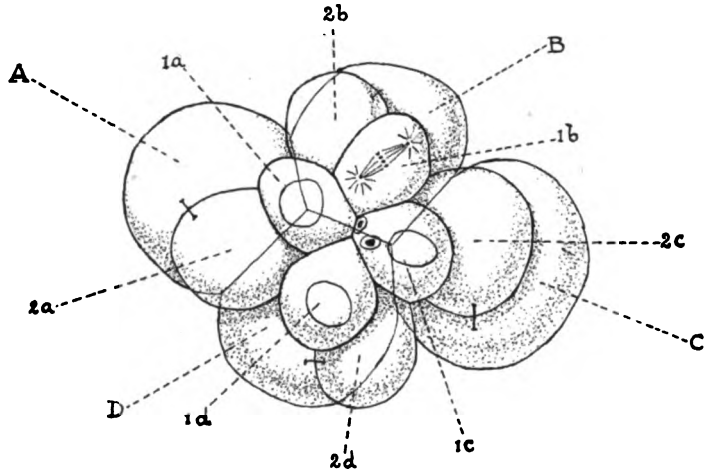

FIG. 15.—The twelve celled stage. The second quartette of micromeres was formed by a laeotropic movement. One cell, 1b, is undergoing segmentation which will result in the formation of a turret cell. × 275.

material made it easy to apply light pressure. A small number of eggs were placed on a slide and covered by a second slide, the amount of pressure was regulated by passing a small rubber band around the slides. The eggs were taken in the one celled stage after the polar bodies had formed; they were left under pressure two hours and the changes which took place during the period were carefully noted. I took pains to see that all of the eggs were forced to segment in an irregular manner. After the pressure was removed the eggs were placed in the aquarium in a bottle which was stoppered with cheese cloth. Although a

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number of experiments of this kind were tried with different degrees of pressure I was not able to get any normal embryos. A few abnormal embryos were reared but they lived but a few days. It hardly seems that it will be possible to get any fruitful results from pressure experiments on the eggs of *H. solitaria* for these further reasons: eggs in the egg masses broken during collection develop abnormally, probably because the sea water gains access to them; eggs laid in imperfect egg masses in the laboratory frequently develop abnormally; occasionally I have

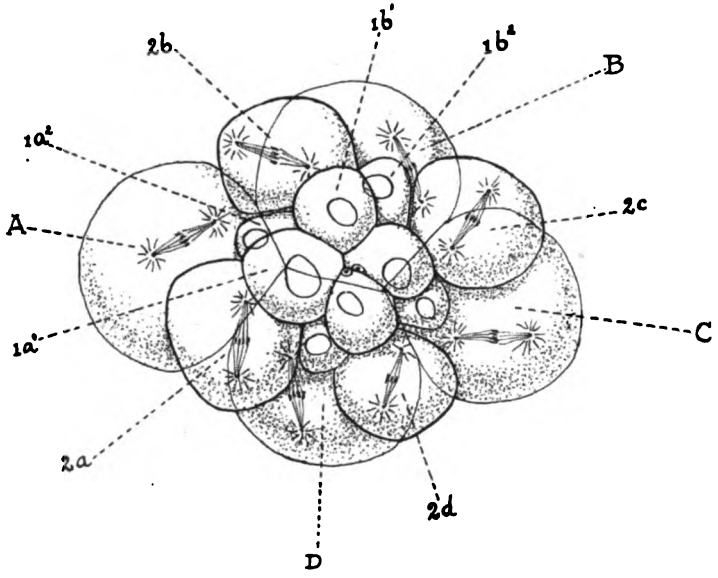

FIG. 16.—The sixteen celled stage. The first quartette of micromeres have given rise to four turret cells, $1b^2$. The third quartette of micromeres is forming by a dexiotropic movement. The second quartette of micromeres is the process of division. × 275.

collected what appeared to be perfect egg masses and found that the segmentation was very irregular, keeping these eggs under observation, I found that they invariably died in a short time; some apparently normal egg masses have been found to contain eggs in all stages of segmentation from the one celled to the thirty celled stage.

In brief then to summarize: The Techtibranch mollusc, first described by Say, and subsequently by Totten, Verrill, and others, should, according to Pilsbry, properly be regarded as *Haminea solitaria*. The dental formula is ∞. 1 ∞. The egg

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laying period extends from the middle of June to the last of August during which time the adults migrate from the deep water into shallow ponds and lagoons. The eggs are laid in a gelatinous mass, spherical in form, attached to eel grass, algæ, stones, sticks, etc. The eggs pass from the one celled stage to the free swimming embryo in seven days. The method of segmentation of *Haminea solitaria* is in close agreement with the other mollusca. No positive results were obtained from attempts to produce abnormal segmentation.

ZOÖLOGICAL LABORATORY, SYRACUSE UNIVERSITY,
November 15, 1903.

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THE SEXUAL ELEMENTS OF THE GIANT SALAMANDER, CRYPTOBRANCHUS ALLEGHENIENSIS.

ALBERT M. REESE.

In the spring and early summer of 1902, the author made strenuous efforts to obtain embryological material for investigating the development of the hellbender (*Cryptobranchus Allegheniensis*). These unsuccessful efforts have been described in an article entitled "The Habits of the Giant Salamander." [1]

In the fall of 1903, another effort was made to obtain the desired material, and a dozen or more live hellbenders were obtained as the result of a trip to the region of the Allegheny River, in western Pennsylvania.

These animals were all about the same size, 45 cm. in length, and were sent by express from their native stream to Syracuse, N. Y.

Upon opening the box, in which they were shipped, after its arrival in Syracuse, a number of eggs were found scattered through the grass that had been placed there to protect the animals during their trip. There seemed to be no difference in the coloration of the males and females, and the only way in which they could be distinguished was by the fact that, in the males, the lips of the cloaca were considerably swollen by the enlargement of an elongated mass of glandular tissue on each side.

In handling one of the ripe females of this lot of hellbenders, the author was bitten on the thumb; this was the only time in which any attempt to bite had been noticed, though many dozen animals had been handled at many different times. The bite was not at all serious, being merely a painful pinch which scarcely broke the skin. In removing one of the females from the box in which they had arrived, an egg, enclosed in its jelly-like envelope, was seen protruding from the cloaca. By gently pulling this extruded egg, it was found that a whole string of eggs could be drawn from the cloaca, without apparently injuring them in the least.

[1] *Popular Science Monthly*, May, 1903.

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Each egg is a spherical yellow body, about 6 mm. in diameter, resembling somewhat the yolk of a miniature hen's egg. It is surrounded by a clear gelatinous envelope, which is arranged in two distinct layers (Fig. 1).

When removed from the gelatinous envelopes, as may easily be done by cutting through the latter with a pair of fine scissors, the egg is seen to be enclosed in a very thin and delicate vitelline membrane which is easily torn in handling.

The yolk, which is apparently evenly distributed throughout the egg, is made up of a compact mass of granules of various sizes (Fig. 2).

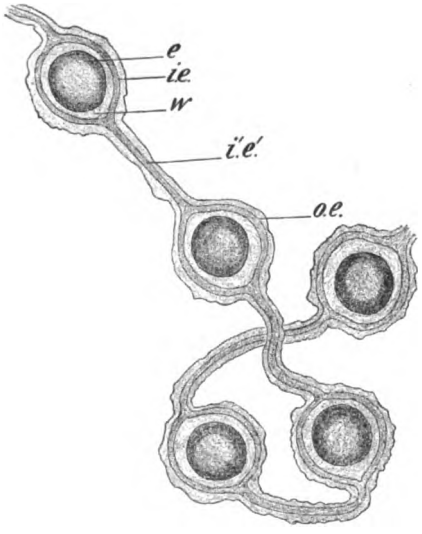

FIG. 1.

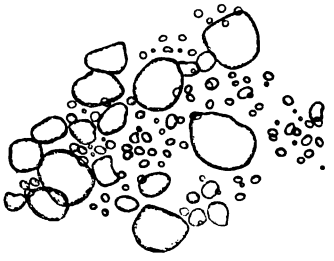

FIG. 2.

The egg is surrounded by a small amount of watery material (Fig. 1, *w*) which is, in turn, enclosed in a capsule of more dense jelly, the inner envelope (Fig. 1, *i. e.*). The inner envelope is continued as a solid, more or less tough cord of jelly (*i'. e'.*) from egg to egg, and binds them together in the continuous strings that have already been mentioned. The distance between two adjacent eggs of the string is usually about four or five times the diameter of the egg, but the elasticity of the jelly will, of course, permit the eggs to be drawn much further apart.

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The outline of the inner envelope is sharp and even, while that of the outer envelope (Fig. 1, *o. e.*) is more or less irregular and uneven. The outer envelope is composed of such transparent jelly that it might easily be overlooked at the first glance. It forms a continuous layer over the entire mass of eggs.

When the unfertilized eggs are left for some days in water, they become very much swollen, by the osmosis of water through the vitelline membrane, and may eventually burst.

There was no apparent swelling of the gelatinous envelopes on coming in contact with water as is described in connection with some other amphibian eggs.

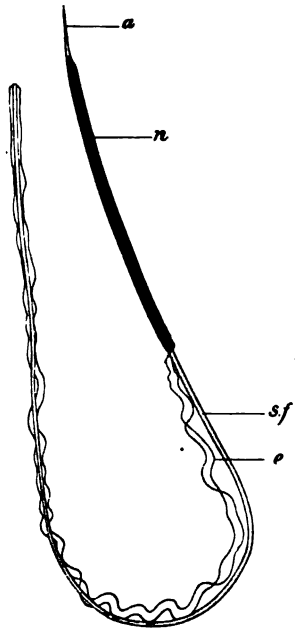

FIG. 3.

Several dozen eggs were obtained from one average-sized female, about two dozen being drawn, without apparent injury, from the cloaca, while the rest were obtained only after killing the animal and opening the body cavity. All the eggs obtained in the latter way were found to be contained in the right oviduct, the ova of the left ovary being nearly all in a very immature condition. Whether or not this was a normal condition, indicating perhaps, a very prolonged breeding season, it was not possible to say.

The spermatozoa were obtained as a milky fluid from the living males by the usual process of stripping, though considerable pressure had, in most cases, to be exerted. They were immediately examined under the higher powers of the microscope, but no motion could be detected, though it would naturally be expected that spermatozoa obtained in this way would show the usual activity of mature spermatozoa.

An attempt was made to artificially fertilize the eggs by putting them into a dish of water into which a great nnmber of spermatozoa had been stirred, but the attempt was entirely unsuccessful.

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No structures resembling spermatophores were discovered, and there was nothing that would seem to give any indication of the method by which the act of fertilization was accomplished.

A single spermatozoön, as seen under a magnification of about 1,300 diameters, is shown in Fig. 3.

Fairly good preparations were easily made by drying them rapidly on the slide, and staining in hæmatoxylin and eosin.

The nucleus, *n*, is very much elongated, so that it makes up almost one third of the entire length of the spermatozoön. It is capped, at its anterior end, by a sharp, gradually-tapering apical body, *a*, which is plainly differentiated from the nucleus proper by the fact that it does not take up the stain to any great extent. No structural details in the nucleus or apical body can be discerned with the magnification used, nor is any middle-piece distinguishable. The tail, which is comparatively stout, consists of a central supporting fiber, *s. f.*, which takes up the stain slightly, surrounded by a transparent envelope, *e*, which does not stain at all. The envelope is usually considerably wrinkled and twisted, probably by the rough method of fixation.

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BUDDING TENTACLES OF GONIONEMUS.

GEORGE T. HARGITT.

While looking over specimens of *Gonionemus* for class work, and being somewhat on the lookout for any cases of variation, etc., my attention was arrested by an unusual appearance of a tentacle. Near the distal end was present a small knob which at first glance appeared simply as a protuberance, apparently without any very definite form or structure.

On further and more careful examination it seemed to me to warrant a careful study. It was somewhat similar to some of the conditions found by Hargitt[1] in his work on the variation of

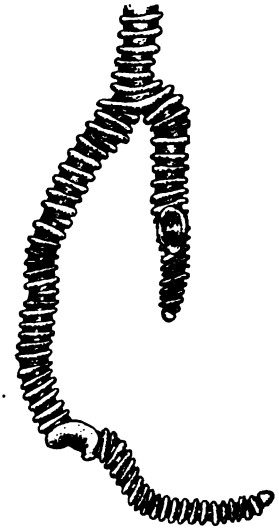

FIG. 1.

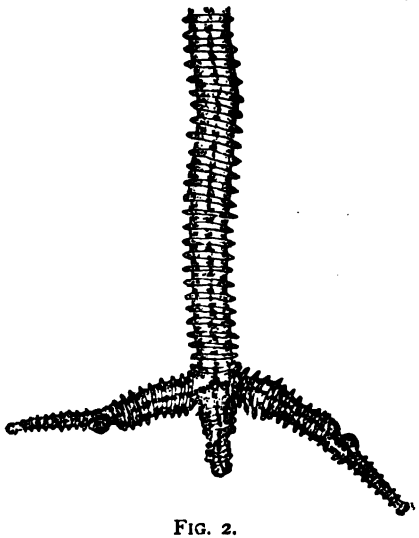

FIG. 2.

this form. The conditions referred to are the presence of bifurcated tentacles. He found a number of tentacles which had double, or in one case triple tips. In all these cases the extra tip or bud seemed to arise either from the suctorial pad or else immediately proximal to it. Fig. 1 represents a rather typical example of bifid tentacle. The specimen is one I found in C.

[1] "Variation among Hydromedusæ," BIOL. BULL., Vol. II., No. 5, 1901.

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W. Hargitt's collection, but which he did not use in his paper on variation. The extra tip is seen to be much shorter than the main tip. Each tip is supplied with one of the suctorial pads, but the shorter one arises from a point considerably proximal to the pad on the main tentacle and directly from the tissue of the tentacle. No sign of injury is present. The appearance of this tentacle suggests the probable result of further growth of the bud shown in Fig. 3, except of course the lack of the pad at the base of the bud. Fig. 2, showing a trifid tentacle, is taken from Hargitt's paper on variation (*op. cit.*). It shows two branches arising from near the end of the main tentacle which seems to be degenerate as mentioned later. The buds here do not seem to arise from the suctorial pad of the main tentacle, which is not shown, but each bud is supplied with a pad near its tip. The knob on the tentacle under consideration, however, had more the appearance of a bud than a bifurcation. This was due chiefly to its small size which rather suggested that it was a very early stage in the formation of an extra tip to the tentacle.

The bud arose from a definite base which presented almost exactly the same external appearance as the normal suctorial pad. This similarity consisted not only in the smooth appearance, due to the absence of the ectodermal ridges found on the other parts of the tentacle, but also in its concave form, and the further presence of a bend or "knee" in the tentacle at this point; all of which are characteristic of the normal suctorial pad (Figs. 3–5). The bud arose from a depression in the base (Fig. 4) due to the cup-like shape of the pad already mentioned.

No external sign of injury was found either in this pad or in the surrounding tissue. The pad was of course not functional as an adhesive organ, another functional one being present nearer the distal end of the tentacle (Figs. 3 and 5). Whether this new pad formed after the beginning of the development of the bud from the old pad, whose functional activity would thus be destroyed; or whether a second pad formed first, and a bud began to develop from the old one (which would not then be necessary) simply as a result of the capacity for regeneration, or rather duplication of parts, inherent in the tentacles, is an extremely interesting question. Of course no direct answer can be

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made without a considerable body of facts before us, coming from actual observations on this particular point; facts which it would be very difficult if not impossible to obtain. It seems to me, however, that we can suggest a probable answer from conditions observed in other cases, which bear more or less directly on this point. A comparison of Figs. 1 and 2 with some of those in Hargitt's paper show that buds do not always arise from pads. Indeed of the six figures of bifid and trifid tentacles shown in that paper and in this, only two show the bud as arising from the pad. This alone would show quite conclusively that there is no necessary or regular connection between the bud

FIG. 3.

FIG. 4.

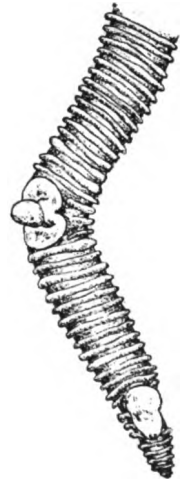

FIG. 5.

and the suctorial pad. This is further emphasized by the fact that I found nine tentacles having each three pads, apparently well developed and functional, and yet there was not the least sign of buds arising from any of the pads. These facts all point directly toward an unusual predisposition to a duplication of organs, and this perhaps offers the most satisfactory explanation of the budding and bifurcation of tentacles.

The bud showed a constriction near its middle region, but did not present externally any signs of annulation, or rather of the ectodermal ridges present on the old tentacle. However, nematocysts were present in abundance. The general appearance of the

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bud is similar to that of the old tentacle, with the exception noted above, and is undoubtedly of the same structure.

Hargitt (*op. cit.*, p. 244) in referring to bifid and trifid tentacles suggested that they might have orginataed is the result of some injury to the distal end of the tentacle. This seemed to be especially indicated by the one trifid tentacle found. In this case there seemed to be a degeneration or atrophy of the median branch, which was probably the end of the original tentacle. From the sides of this tentacle two branches arose opposite each other which were considerably longer than the median tip (*cf.* Fig. 2). He says concerning the cause of this: "The degenerating middle tip would very naturally suggest the probability that an injury might have been the predisposing cause of the secondary tips; on the other hand, it must not be overlooked that in each of the other specimens with double tips no such cause seems at all evident."

It was with the thought of trying to determine whether there was any sign of injury which might have influenced the formation of a bud in that region, as well as to determine the histogenic changes involved in its formation, that I was led to undertake a careful study of this budding tentacle.

The tentacle was stained in toto with borax carmine. Sections were cut transversely across the tentacle, thus making the sections of the bud longitudinal.

Fig. 6 represents a section of the entire tentacle showing the bud in its general relations. The entoderm of the bud is seen to be directly continuous with the entoderm of the tentacle. The bud is solid with the exception of a cavity at the distal end and there is no connection between this cavity and the cavity of the tentacle. It will be noticed, however, that the cells are arranged more or less definitely in two rows with the dividing line quite distinctly marked in the proximal region, as though in further growth these would pull apart and thus connect the distal cavity of the bud with the cavity of the tentacle. On either side of the bud are masses of the rather dense tissue which makes up the suctorial or adhesive pad already mentioned. This tissue resembles very much muscular tissue rather than glandular tissue, suggesting that the pad acts by virtue of its muscularity, rather than by

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means of a secreted adhesive substance as suggested by Perkins.[1] At the edges of this pad is present the collar-like expansion of the ectoderm which is characteristic of this structure normally. Indeed, the shape, size and contents of these cells, as well as their method of staining and general appearance, is almost exactly the same as in the normal pad. Thus there seems little doubt that there was originally present here a functional suctorial pad. So

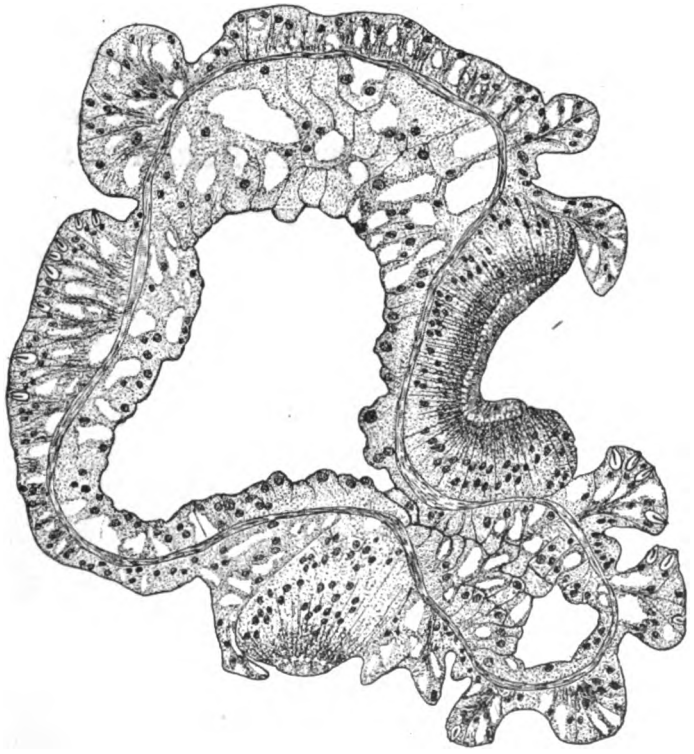

FIG. 6.

far as we can tell from the sections the pad might have formed after the bud began its development, though this is very unlikely, since in such case the function would be quite limited. Indeed the pad would lose its power of functioning normally if the bud increased in size to any extent. The ectoderm of the bud is thrown into the folds or ridges, which are characteristic of the normal tentacles, and nematocysts are limited to these ridges (*cf.* figures).

[1] "The Development of *Gonionema Murbachii*," *Proc. Acad. Nat. Sci.*, Philadelphia, p. 764, 1902.

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Figs. 7, 8, 9 show only the bud and the tissues immediately adjoining. The same general features already noted are also seen here. In Fig. 7 the muscular pad on each side of the base of the bud is nearer the same size than in Fig. 6. The relation of the entoderm of the bud and tentacle is shown in about the same way, but the arrangement of the bud entoderm into two rows is not so distinctly marked. In Fig. 8 this arrangement of entoderm is scarcely indicated, the cells being more or less massed together and not showing any apparent regularity. The character of the tissue of the muscular pad is shown better as are also the ectodermal ridges. The cavity at the distal end of the bud

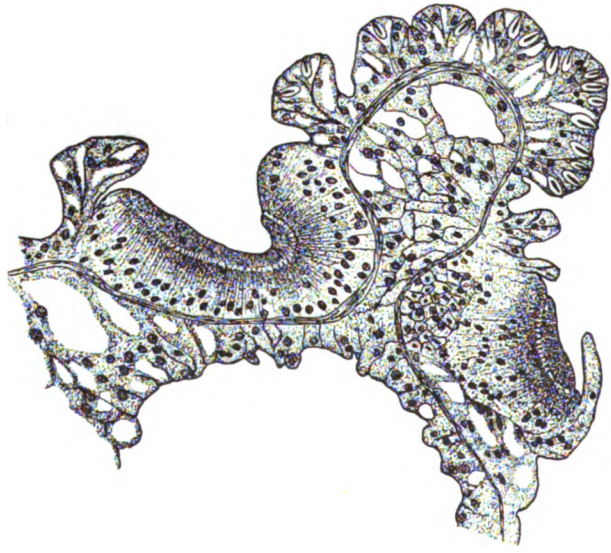

FIG. 7.

is larger than shown in the other figures. In Figs. 8 and 9 the muscular pad is shown on only one side of the base of the bud, showing that the bud is not completely surrounded by it, a feature also indicated in Figs. 3 and 5, where the pad is seen to have a notch or sinus on one side. Fig. 9 represents a section cut one side of the long axis of the bud, so that the entoderm of the bud and tentacle is not continuous.

It will have been noticed that in all the figures the bud is solid with the exception of a cavity at the distal end. Perkins (*op. cit.*, p. 785) referring to the development of the normal tentacle states

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that it is at first solid, but that later "the cavity of the circular canal is drawn into it." "The entodermal cells, arranged radially about the central axis, thicken until they are forced away from the center and a tubular cavity is left." This process, he states, begins at the proximal end and the cavity is gradually "carried out along the axis of the tentacle toward the tip." In this bud there seems to be present the cavity at the distal end so

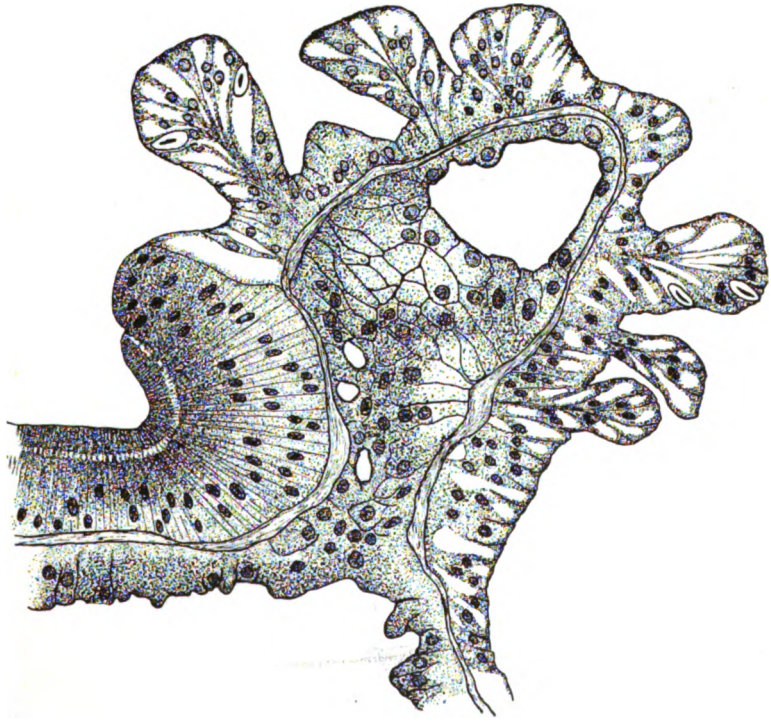

FIG. 8.

that the method just mentioned does not apply, at least not wholly. In Fig. 6 the cells are evidently arranging themselves in rows next the supporting layer, with their edges meeting near the center, suggesting a drawing away from the axis and a formation of a cavity (as Perkins suggests) connecting the cavity of the tentacle with the cavity already formed in the bud. Fig. 9, however, would seem to indicate a somewhat modified process. The cell outlines are not distinct, so that their arrangement cannot be definitely determined, but in the central part of the core are a

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number of irregular cavities. This suggests the possibility of these cavities enlarging and running together, the cells at the same time taking up a regular position next the supporting layer, and thus the cavity of the bud being formed. In neither case, however, would the process necessarily begin at the proximal end. Furthermore, it is not quite certain just how the cavity at the distal end of the bud forms, or why it should form so early and not involve the proximal portion of the bud.

In regard to the early method of formation of the bud little can be suggested since it has developed beyond the initial stage. It can be said, however, that not the slightest trace of injury was found, which might be a predisposing cause. Alb. Lang [1] from

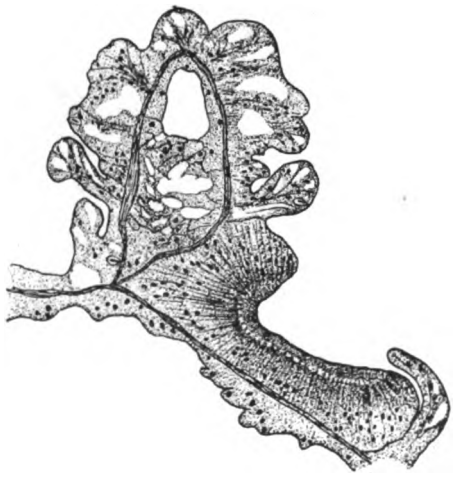

FIG. 9.

his work on budding in *Hydra*, *Eudendrium* and *Plumularia* tried to show that the bud originated from the ectoderm entirely, that the previous view that both layers were active could not be maintained ; ectoderm cells migrated through the supporting layer and formed the bud entoderm, the old entoderm being absorbed. Seeliger [2] and Braem,[3] however, working on *Hydra*, *Eudendrium*, *Plumularia*, *Obelia* and *Sertularella* claimed that Lang's results were entirely incorrect and the conclusions drawn from them

[1] *Zeitschr. f. wiss. Zool.*, Bd. 54, 1892, pp. 365–385.
[2] *Zeitschr. f. wiss. Zool.*, Bd. 58, 1894, pp. 152–188.
[3] *Biol. Centralbl.*, Bd. 14, 1894, pp. 140–161.

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not warranted. They found dividing cells in the entoderm as well as in the ectoderm, a condition which Lang did not find. They found no trace of ectoderm cells migrating into the entoderm even in the earliest stages, of the two layers running into each other, of the entoderm being pushed aside and absorbed. Many others also have questioned Lang's claims and maintain that results obtained in many species of hydroids, as well as in other forms of animal life, confirm the old view. My own work on hydroids [1] confirm these results of Seeliger, Braem and others. In working on the regeneration of *Tubularia crocea*, *T. tenella*, *T. larynx*, *Eudendrium ramosum* and *Pennaria tiarella* particular attention was paid to structures which form either wholly or in part by budding. Sections made through these buds in all stages showed none of the features claimed by Lang, such as migrating entoderm cells, dissolution of the supporting layer with the accompanying disappearance of sharp contrast between the two layers, and absorption of the entoderm. Often, usually indeed, the entoderm seemed to be the layer most active in this process. Mitotic division was observed in both ectoderm and entoderm though not abundant, reasons for which are discussed in the above-mentioned paper. Amitotic division was also more or less prevalent in both layers. Perkins (*op. cit.*, p. 784) referring to the formation of the tentacle in *Gonionemus* says the three layers, ectoderm, entoderm and supporting layer "are pushed out somewhat in the growth of the tentacle, the region of greatest activity being the endodermal layer, where the core of the tentacle is formed by a rapid outgrowth of the cells of the body wall accompanied by multiplication of these same cells." He further states that there is not even an initial thickening of the ectoderm in the region where the tentacle is to appear. In regard to budding in the larval form likewise, he states that the ectoderm and entoderm cells divide, the entoderm pushes out gradually, the ectoderm growing so regularly as to cover it with a layer of constant thickness. Thus it has been conclusively proved that budding in many hydroids, as well as in the formation of the tentacle and in other buds of *Gonionemus*, involves both layers, and cells in both layers increase rapidly by division. This would entirely dis-

[1] *Arch. f. Ent-mech.*, Bd., XVII., Heft 1, 1903, pp. 64–91.

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credit the universality of Lang's claims, even if they held true for a few forms.

Since this is the method of the formation of the tentacles, and of buds in other regions of *Gonionemus*, we may safely assume that probably the same process would be active in the formation of the bud from the tentacle, or at least to a great extent. Greater support is given this assumption by the fact of the presence of mitotically dividing nuclei found in the entoderm of the bud. They were not definitely determined in the ectoderm though doubtless present.

I am glad to acknowledge my indebtedness to my father, C. W. Hargitt, for permission to reproduce Fig. 2, which appeared in his paper on "Variation among Hydromedusæ," and also for allowing me to examine his collections of *Gonionemus*.

ZOÖLOGICAL LABORATORY, SYRACUSE UNIVERSITY,
January 18, 1904.

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REGENERATION IN RHIZOSTOMA PULMO.

BY
CHARLES W. HARGITT.

WITH 6 FIGURES.

I. INTRODUCTORY.

The several experiments, of which this paper presents a resumé, were conducted during the early summer of 1903, at the Naples Zoölogical Station, while occupying the table of the Smithsonian Institution, for the courtesy of which it is a pleasure to express my obligations.

The primary object of the experiments was to test the regenerative capacity of the Scyphomedusae and to institute certain comparisons between these results and those obtained by similar experiments previously made upon the Hydromedusae. So far as I am aware no similar experiments have been made upon the Scyphomedusae with the definite purpose of testing this particular aspect of their physiological constitution. Romanes in his experiments upon " Primitive Nervous Systems," '85, has recorded incidentally the fact that certain mutilations of medusae are promptly healed, but gave no details. Eimer, '78, has also carried on similar experiments and with the same general purpose of testing the character and distribution of nervous centers, but makes no reference to the matter of regeneration. And quite recently Uexküll, '00, has likewise reviewed these experiments of Romanes and Eimer and carried them somewhat farther than they had done. But while arriving at somewhat different conclusions, drawn from a series of experiments in some features coincident with those to be described now, he makes no reference to any regenerative processes, devoting attention almost exclusively to the movements, specially those of rhythmic character, and seeking physical explanations of them.

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The earlier references of Haeckel to the capacity of larvae of certain medusae to regenerate entire organisms are likewise indefinite. Morgan in referring to the subject in his recent book on "Regeneration," '01, merely remarks that among Scyphozoa 'the jelly-fishes belonging to this group have a limited amount of regenerative power."

I very much regret that an unusual scarcity of material compels me to leave several points somewhat less fully considered than is desirable, but I trust they are not of sufficient gravity to seriously mar the general value of the results as a whole.

In one respect this scarcity of material, making necessary successive experiments on the same specimen in many cases, proved fortunate rather than otherwise, since facts of importance were thus brought to light which might otherwise have been overlooked. Some of these will be referred to specifically in another connection.

II. EXPERIMENTAL.

The experiments were performed upon Rhizostoma pulmo, one of the most common of the Mediterranean medusae. Both in size and vigor this medusae affords one of the most satisfactory forms for experimentation which has come under my observation. It seems likewise to suffer less under the somewhat artificial conditions of the aquarium than any other which I have had occasion to use. As compared with Aurelia and Cyanea of New England waters it is incomparably superior in every way, but particularly in its ability to thrive for weeks in an environment which would prove fatal to the others in as many days. With the single exception of Gonionemus I know of no other medusa which affords so good a type for this sort of observation and experimentation. It was not unusual to have specimens under direct observation in the ordinary aquaria of the laboratory rooms for from four to six weeks and without apparent deterioration, even in some cases under the severe tax of extensive mutilation made necessary by the experiments to which they were subjected. It should be stated however that as a rule younger and smaller specimens proved much better than those of larger size; the latter, on account of

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their greater mass, are inclined in most cases to sink toward the bottom of the tanks, where after a time certain disorganizing influences appeared to set up pathologic conditions which seemed to deplete their vigor and at the same time render their regenerative processes less satisfactory.

The experiments were directed to three ends, namely to determine: 1, The capacity of the medusae to reproduce lost parts, or to recover from such injuries as might ordinarily happen to them in a state of nature, such as the battering effects of waves, the injuries inflicted by enemies, etc.

2, The comparative powers of the various regions to regenerate, or in other words, the relation of the regenerative capacity to liability to injury.

3, The capacity to regenerate such highly specialized organs as rhopalia, or other sensory structures.

The experiments included specimens of sizes from about 20 m/m to 125 m/m in diameter, and while all proved to have unexpected powers of regeneration those of medium size, from 40 to 70 m/m, proved very much more satisfactory than those of larger size both in convenience and in their promptness in responding to the several sorts of operations, and they apparently were more healthy and vigorous during the progress of the experiments than were those of larger size. Those having a size of 100 m/m or more in diameter proved to be much less prompt in regeneration and, as will be seen in the records of experiments, were much more liable to deteriorate or utterly collapse than were the smaller specimens. This is only what might be more or less expected, and is quite in keeping with observations on other classes of organisms. The same tendency was more or less evident in specimens on exhibition in the public aquarium in which of course no mutilations or similar injuries had occurred. In this connection may be noted a somewhat anomalous pathological phenomenon observed in large specimens both in the exhibition aquaria and in the small aquaria during the course of experimentaton, namely, the appearance of whitish blotches, or patches of disintegrating tissues at various places on the exumbrella of the animal which sooner or later affected its health and general behavior.

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The matter will be referred to in further detail in another connection and some reference made as to its probable significance and cause.

In all cases the primary experiments were made as soon as possible after the medusae were brought into the laboratory. I have said the *primary* experiments. This refers to the fact already alluded to, that in several cases experiments were variously repeated upon the same specimen. This was in part for the purpose of testing the conclusiveness of preceding experiments, and in part owing to the fact that there was an insufficient supply of material to serve the demands of the course of experiments under way. Details as to these aspects will be given in connection with the several experiments described.

The first experiment was made upon a large specimen, and in order to determine at the outset whether the earlier observations of Romanes and others, that complete removal of the marginal sense organs resulted in complete paralysis of the medusa, these organs were carefully removed by means of triangular incisions as indicated in Figure 1, *a*. The results were substantially confirmatory of the earlier records, the medusa becoming more or less passive, except for an occasional single contraction at very irregular intervals. This experiment was made on May 11, and the following series of observations will suffice to show the general course of events. It should be added in this connection that along with the excision of the rhopalia several other marginal excisions were made, and that three of the oral arms were cut off close below the region of the gastric enlargement. The aspect of the specimen on the next day was practically the same. While there was an occasional contraction of the bell accompanied by certain movements of the body, there were no indications of rhythm.

May 13th.—The medusa, while apparently in perfect health and vigor of general functions, was still unable to originate any definitely rhythmic movements, though responding to various mechanical stimuli, such as a strong current of water from the tap, or the touch of a glass rod. At various times during the day there was evident a rather marked tendency toward spontaneous movements, and occasionally something very like a rhythm,

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several contractions following each other in regular succession, though never continuing beyond three or four pulsations.

May 14th.—The medusa, while still more or less passive as before, was yet apparently recovering more of the power of spontaneity, several pulsations occurring at more frequent intervals, but these were not of sufficient vigor to produce any locomotion.

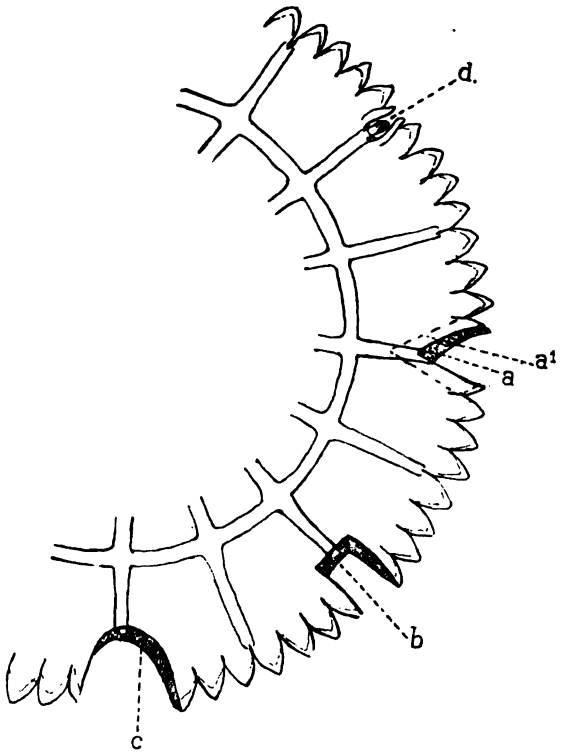

FIG. 1.

Diagram showing methods of excising rhopalia.

a, usual triangular excision; a^1, excision of larger mass; *b*, rectangular form of excision; *c*, circular form of excision; *d*, form of rhopalium and lappetts.

May 15th.—During this and the following day there was an apparent relapse of the medusa to the condition of the first day. There was also less vigor apparent, such stimuli as those referred to above producing but slight effects. This condition continued during the 18th, 19th and 20th.

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May 21st.—The medusa seemed to have recovered the vigor or tone to which reference has been made above. There was also a very evident rhythm in the contractions, often as many as ten or more regularly recurring pulsations occurring at irregular intervals during the day. As before, however, they were not of sufficient force to secure the locomotion of the animal. The same condition was observable during the following day.

May 23d.—There was again a marked decline in both vigor and general tone of the body, which showed evident signs of degeneration. This condition continued during the following day, and on the morning of the 25th the medusa was found to have died during the preceding night.

Upon careful examination it was found that wherever tissue had been mutilated or excised there had been a definite healing of the wounds and in the case of the oral arms there were indications of new growth. I was not able to distinguish that there had been any regeneration of the sensory organs, and this will appear somewhat surprising in the light of the following experiments. Whether there had really been no regeneration at all, or that I had overlooked the new organs, or whether they may have disintegrated during the night following the death of the medusa I am unable to say. Certain it is, however, that if regeneration had gone forward as markedly as in the following cases one could hardly have failed to distinguish it. I am inclined to believe that the paralysis following the total removal of these organs may have served to delay or inhibit active regeneration.

The next series of experiments differed materially from the former, particularly in that care was taken to retain certain of the rhopalia in order to insure continued activity of the organisms during the progress of the experiment. The number of rhopalia retained varied from one to eight, the latter case serving as a means of testing the relative influence of these bodies on the behavior of the animals and the rate of regeneration.

On May 12th several specimens, averaging only about half the size of the preceding, namely, about 50 m/m in diameter, were experimented upon. In the first one all the rhopalia were retained, but marginal notches were made of varying sizes between the

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sensory bodies, and several of the oral arms were excised. In other specimens a varying number of the rhopalia were excised, and in one case all the oral arms were cut off close to the gastric enlargement and on one side including a portion of this organ itself.

I shall not undertake to transcribe in detail the records of each day, but give rather summaries of results as briefly as is compatible with clearness, trusting that nothing of importance may be sacrificed in the attempt to bring the records within as brief compass as possible.

One of the first effects distinguishable in these and following experiments was the evident quickening of the pulsations of the medusae by the process of excision of the organs, or similar operation. Not only was the rate of the rhythm greatly increased, passing from about seventy pulsations per minute as an average for medusae of this size, to ninety, or even one hundred per minute. And this rate continued during the entire day, or at every observation, which was quite frequent, and well on into the second day, when the rate fell to ninety and later to eighty; but it was not till the third day that the rate had fallen to the normal of seventy per minute. An examination at this time showed an evident healing of the wounds and some signs of regeneration. Had this been restricted to the sensory bodies it might have been interpreted as signifying some important relation of these organs to rhythmic activity, but the fact that similar effects were produced upon specimens which had not been deprived of their rhopalia would sufficiently negative such an inference.

Eimer, '74, had noted such an effect following a division of medusae, particularly those which had been divided into halves or fourths, and had undertaken to show that it was chiefly an expression of the reduced size of the organism due to its division, citing the normal rhythm of specimens of varying size as strongly suggesting such an inference.

Romanes, '85, however, was not able to confirm Eimer's contention either in reference to matter of fact or the cause assigned. Romanes, while citing the variation as to the rate of rhythm in

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specimens of similar size, is inclined to emphasize what he terms the prepotent influence of certain of the lithocysts (rhopalia) in coördinating the rate of movement, and the presence or absence of such prepotent organs in the portions of medusae under examination.

Forbes, '48, had long previous called attention to the fact of these quickened movements under the influence of various stimuli, citing particularly a result of an experiment which he had made of a similar character to those which I have cited above. In an experiment in which he had, as he expresses it, "paralyzed one half of the animal" by cutting out the rhopalia from one side, he finds "that the other half contracted as usual, though with more rapidity, as if the animal were alarmed or suffering." He remarks farther that "all medusae when irritated become much more rapid in their movements and contract or expand their disks or bodies in a hurried and irregular manner, as if endeavoring to escape from their persecutors." (Naked Eyed Medusae, p. 3.)

While in certain details the conclusions of Forbes may be questioned, of his general observations as to matters of fact there can hardly be doubt. Furthermore, whether the suggestions of either Eimer or Romanes are more than approximate guesses, the later observations of Uexküll have rendered doubtful. So far as my own experiments have gone they hardly touch the problem of the cause of such reactions. We may safely conclude that, in any case, they are of the nature of responses to any continued physical stimulus, such as the experiments under consideration certainly were. With the healing of the wounds there would of course ensue a decline of the irritation, which in turn would be followed by a return to the normal rate of rhythm.

On May 26th, or two weeks following the operation, the medusae had measurably regenerated all the excised organs. The notches cut in the umbrella margins had grown out to complete the normal symmetry and there had been developed in the areas the characteristic purple pigment, differing from the color of the uninjured portions only in its intensity. The new rhopalia were apparently normal in everything save size and pigmentation.

It is rather noteworthy that in these experiments certain of the

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organs which among the Hydromedusae are most promptly regenerated are here among the most slow to develop; such, for example, as the oral arms and gastric lobes. The fact that in the rhizostomous medusae these organs have no very active function in the capture of food might apparently afford some plausible reason for this difference in the rate of regeneration. In Gonionemus the gastric and oral organs are among the most prompt in regeneration, and are, of course, also among the most important in the functional activities of the animal. That this, rather than liability to injury, should be a predisposing factor in regeneration would seem to be confirmed in the case of Rhizostoma, for as will appear in later experiments there seems to be no good reason to suppose that the liability to injury, to which these organs are constantly exposed, has anything to do with the capacity for rapid or perfect regeneration.

Additional experiments were begun on May 28th and 30th. In this series the specimens varied in size from 20 to 60 m/m in diameter. As remarked above there was in these cases the same degree of promptness in the responses, which was markedly in contrast with that shown by specimens of considerably larger size, but in the present cases there was also apparent a somewhat less favorable response in the very small specimens. This fact considered in connection with the difficulty of operating easily upon small specimens, emphasizes the value of animals of medium size for such experiments. This conclusion was emphasized throughout the entire course of experimentation.

In part of the specimens of this series only three rhopalia were excised, in others four, in others five. In some the rhopalia were all removed from one side, while in others only alternate organs were removed. In some specimens the same order was observed as to excision of mouth arms and other similar operations. One of the specimens of the series had only one full-sized mouth arm, while the others were in what seemed to be various stages of regeneration. As is well known these organs among medusae of this type are among the most open to accident from attack of fishes or other predatory enemy. The specimen under consideration would seem to confirm the results of these experiments that

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these organs are readily regenerated, and that in a state of nature as well as under the artificial conditions of the laboratory. An examination made with the hand lens on June 2d, or only four or five days following the operation, showed the first indication of regenerating rhopalia. As the organ first makes its appearance it is a very minute papilla-like body, and in these cases at the inner, or upper edge of the notch made by the incision. Examined under the compound microscope the papilla appears as a minute, solid bud growing out from the terminal region of the radial canal, though it does not at first seem to be a direct outgrowth of that organ. Very soon, however, there is established a direct connection with the canal, and it is quite easy to distinguish the circulation of the gastric fluid in the little bud, which becomes definitely vesicular, as shown in Figure 2. The growth of the organ, after its vesicular stage is established, is quite rapid and there can soon be distinguished the thickening of the terminal portion to form the lithocysts. Coincident with this stage of development there is discernible the development of the new hood and lappets, accessory organs, and as will be shown in connection with a study of the histology of these organs, the corresponding development of the so-called olfactory and ocellar pits.

In connection with the present series the following experiments were made with a view to demonstrate that, not only in form but in function, the new rhopalia were perfect organs. From one of the specimens just described in which three rhopalia had been originally excised the other five were excised on June 5th, or seven days after the original experiment. If the three regenerated organs had not yet attained to functional utility the effect of removing the others would, of course, result in the typical paralysis, as in the first experiment already described. As was anticipated, the careful removal of all the rhopalia except the three regenerated ones did not in the least interrupt the normal rhythm or activity of the creature, save to act as a stimulus to quicken it, as already cited in connection with a previous series. This experiment was repeated upon several others of this as well as subsequent series, and always with the same results, except in a single case which may as well be cited in this connection, though coming under later experiments.

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In this case the original operation had removed six rhopalia, leaving but two. Soon after the appearance of the new rhopalia, but before they had begun to approach complete development, or before there was any indication of the presence of lithocysts or pigment, the two original organs were carefully removed, and in this case with what might likewise have been anticipated, namely, the complete inhibition of the normal rhythm and the consequent paralysis of the organism. This inhibition continued during the

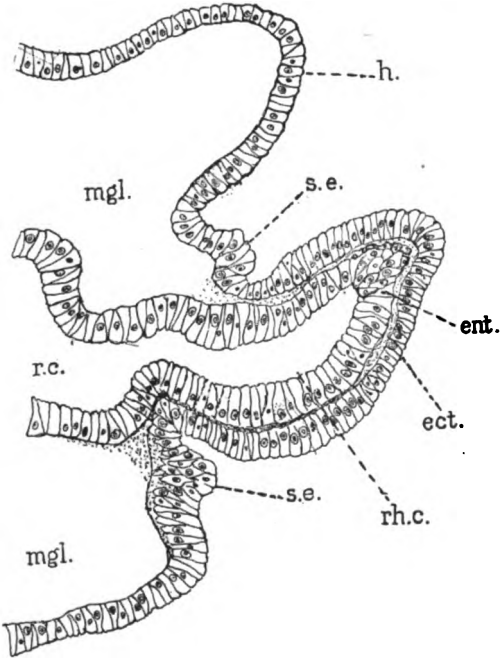

FIG. 2.

Section of rhopalium in early stage of regeneration. ect, ectoderm; ent, entoderm; h, hood; mgl, mesogloea; r. c., radial canal; s. e., sensory epithelium.

following two days. With the continued development of the new rhopalia activity was recovered, though, owing to the interposition just at this juncture of an unhealthy condition of the medusa, it failed to entirely recover the usual vigor or tone which the others had shown.

These experiments, abundantly corroborated by subsequent ones, leave no shadow of doubt, it seems to me, as to the capacity of

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these organisms to regenerate in the last detail one of the most highly specialized organs known among Coelenterata. This will be shown more fully in connection with the later account of the histology of the regenerated organs.

Other series of experiments, continued to June 20, while varied in some aspects of detail, were of substantially the same character and with results quite similar to the preceding.

In several of the experiments care was taken to so modify the form and extent of the excised portions as to secure evidence as to the influence of contiguous tissues or parts upon the regenerating organs. In Figure 1 is shown, for example, several aspects of the mode of excising the rhopalia. For the most part the excision was in the form of a triangular cut from the margin inward toward the radial canal, as shown in the figure. The dotted line a^1 will show also in the same connection the occasional extension of the cut to include twice the usual mass. In Figure 1, *b*, will be seen another form of operation. In this case the portion cut out was

FIG. 3.

Twin rhopalia regenerated in place of the single original one.

rectangular instead of triangular, as in the former. The mass excised in the operation also varied as before. At *c*, in the same diagram, may be seen another form of excision in which the cut was circular instead of angular, as in the former cases. It is interesting to note that, so far as I was able to determine, the form of the excision had no perceptible effect upon the form or rate of regeneration. In the case of the rectangular or circular excisions the new organ appeared in its typical place at the median position of the upper portion of the notch. In the case of the large or small portions excised in the triangular cuts not the slightest difference could be distinguished. With the exceptions of some two or three cases to be considered, there was not the

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slightest evidence of any deviation from the exact position occupied by the original organ.

The apparent exceptions referred to are as follows: First, that in at least two cases twin rhopalia were developed instead of the single original one which had been excised. This is well shown in Figure 3. Second, that in one case two rhopalia were regenerated instead of the one originally excised, but unlike the preceding, they appeared at different points — one in the usual position at the upper angle of the notch, the other at the lower, or marginal portion of the notch, as shown in Figure 4.

The mere fact of the occurrence of double rhopalia during regeneration instead of single ones is not of itself particularly remarkable, for the occurrence of such features is not an unusual one in a state of nature, both ephyrae and adult medusae being occasionally found with such double organs. Some further inquiry should, however, be directed to the peculiar position in which the organ noted in Figure 4, at *a,* occurs, namely, at one side of the notch and near the margin instead of the usual position. On the assumption that these organs are of sensory function and correlated with marginal nerve centers it might be

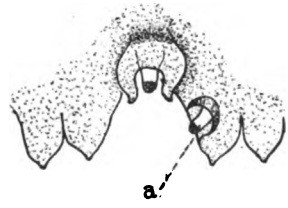

FIG. 4.
Two regenerated rhopalia; a, near the margin.

thought that in regeneration they would be likely to occur in close relation with such centers, and that the case under consideration might be thus explained. The fact is very clear, however, that such is not the case with the vast majority of the experiments where apparently the relation of nerve centers had nothing whatever to do with their position in regeneration. And when furthermore we reflect that these are not nervous organs in any true sense, either in their origin or development, though possibly correlated with

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some sensory function, it must be more or less evident that such an explanation of the single case cited would hardly hold.

Nor would it perhaps be more satisfactory to appeal to what has been designated as polarity in explaining either series. The occurrence of the organs in conjunction with the radial canals and their apparent differentiation from terminal portions of these structures would seem to afford a much more probable explanation of their regeneration at these apparently predetermined positions. And may we not find in this view a simple explanation of the occurrence of the anomalous case referred to in Figure 4, *a,* for we find near the margins a more or less complex network of anastomosing canals, the presence of one of which may have been the inciting cause of the development of a sensory body at this particular point.

It is interesting to note in this connection that no appearance of heteromorphism occurred during the entire series of experiments. This feature I have referred to in a previous paper, '97, in connection with similar work on Hydromedusae. On the assumption that these organs are metamorphosed tentacles we migh naturally look for heteromorphic phenomena similar to that recorded among the crustacea, in which occasionally instead of an eye an antenna develops. Nothing of the sort, however, occurred. There seems in every organ and tissue a remarkably inflexible physiological constancy. This is the more remarkable when contrasted with the highly flexible character of the polyp phase of the group among which are found the widest range and variety of heteromorphism.

The fact is not overlooked that Rhizostoma is devoid of tentacles, which might be assumed as sufficient reason why heteromorphism of this sort was not manifested. The fact remains, however, that its polyp has the typical tentacular equipment, and that in its metomorphism they are resorbed and possibly take the usual course, some of them contributing toward the formation of rhopalia. It might be an interesting problem to determine in detail just the extent of this supposed metomorphism of the polypal tentacles into rhopalia. May it not be possible that the supposed metamorphism is in reality a resorption and that only,

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and that the rhopalia are essentially independent developments such as are found during the process of regeneration? I merely raise the suggestion as it has been forced upon my attention in course of these experiments. It seems worth farther investigation.

In this connection may be briefly described a phenomenon which only came under critical observation late in the course of the experiments, and which for lack of material it was impossible to follow out to conclusive results. Among the last of the series two large specimens were operated upon as follows: In the first all but one of the rhopalia were excised, while in the second all but two were removed. In both cases there was distinctly noticeable an aberrant, rotary sort of swimming movement, the animal revolving in an irregular circle, instead of directly forward or upward as is usual. Examination showed that this inclination of the body in swimming was constantly in the direction of the remaining rhopalia, which would seem to suggest that perhaps they functioned something after the nature of equilibrium organs. I do not recall that this feature has been referred to by the investigators previously cited, and very much regret that it was not practicable for me to carry out such additional experiments as would have afforded more definite conclusions. It must suffice to merely mention the matter, hoping that at some time someone may be able to secure definite conclusions by extended experiments not only upon this medusa but perhaps on others as well.

III. ABNORMALITIES.

In connection with observations upon several specimens which had become degenerate or perhaps pathologic, resulting from unfavorable conditions of some of the aquaria, or perhaps in some cases due to the depleting effects of the experiments, as in the case of the first experiment cited in this paper, occasion was taken to examine somewhat in detail the observations and experiments of Uexküll and to compare cases coming under my own observations during the course of the experiments.

In one specimen which had shown evident decline of vigor and upon which there appeared certain exumbrellar blotches or cor-

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rosion patches, similar to those mentioned in the earlier portion of this paper and comparable in general aspects to cases mentioned by Uexküll, it was found that after all the rhopalia had been removed the specimen yet exhibited certain convulsive contractions which at times simulated an irregular rhythm. I therefore undertook to repeat several of this observer's experiments as to the effects of certain chemical stimuli, specially that of common salt, NaCl. Small crystals of this salt were carefully placed on definite parts of the sub-umbrellar musculature, and I was able thereby to confirm in the main his results. There was a very evident white coloration of the adjacent tissues, and this was followed by a more or less definite, though somewhat irregular, rhythmic contraction of the umbrella which continued for perhaps five minutes. The experiment was repeated several times and upon different specimens and with usually similar results, though differing as to vigor or continuity.

Uexküll had concluded that the recovery of a similar rhythm in specimens upon which he had experimented by excising the rhopalia was due, not to any direct restoration of nervous or other normal equilibrium, but to certain pathologic conditions which had intruded themselves, and among which he was specially impressed by these corrosion abscesses or disease patches, to which reference has been made. Doubting whether an agent of this sort, affecting particularly the exumbrella, could have any very definite importance as a center of stimulus, it occurred to me to vary the experiment by applying the salt to the exumbrellar region instead of the musculature of the sub-umbrella, and though variously repeated the results were uniformly negative in character, no conclusive responses of any sort being obtained. Nor was there observed any of the whitening effects which were so evident in the previous experiments. We may conclude, it seems to me, that the effects produced by the salt in arousing a simulated rhythm of contraction was due to the direct action of the substance on the musculature itself, and not to any general effect produced upon the coördinating centers of the medusa. These stimulating effects of sodium chloride upon muscular tissue are too well known to call for any special mention in this connection.

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It would seem, therefore, that in the light of these facts one may well question the validity of Uexküll's conclusions, or rather inferences. The mere presence of whitish blotches on an organism would hardly justify, without the most conclusive demonstration, the inference that the presence of similar effects produced by some reagent proved them identical or even analogous. That there may have been certain pathologic conditions operating upon these medusae of which the whitish blotches were in some respects expressions may have some measure of probability. But that these blotches were in themselves the inciting stimuli giving rise to the simulated rhythm must be regarded as doubtful, if not indeed, highly improbable. Such a conclusion could hardly have been suggested had it been observed that the same whitish blotches are not unusual on specimens which have been for some time in aquaria. Moreover, their presence on such specimens has not in the least, so far as my own observations have gone, served to introduce any variation of the normal rhythm, a condition which might not be unusual on the assumption of these disease patches becoming sources of abnormal stimuli, and thereby introducing erratic or conflicting factors into the physiological processes of the organism. It is well that attention should be directed to disturbing conditions of this character in order that undue weight be not given to a single factor in determining so important a problem. On the other hand it may be quite as important that in discrediting one conclusion there is not substituted another of even less value.

One might be tempted in this connection to go somewhat out of the way to consider Uexküll's conclusions as to the purely mechanical function of the rhopalia in relation to the rhythmic action of the umbrella of medusae. If they might be supposed to act after the fashion of the clapper of a bell, using his figure of comparison, in the case of such medusae as Rhizostoma what explanation shall we have for the identical rhythm exhibited by many other medusae entirely devoid of rhopalia or any equivalent organ? Many other objections will immediately arise when one reflects upon the very different histological conditions of structure found in these organs in various medusae, but to take up any one

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of these and other phases of the problem would lead too far afield, and we must satisfy ourselves for the time by the reflection that while such speculations are interesting as well as ingenious they are far from demonstrations.

IV. HISTOLOGY.

A brief study of the histology of the regenerated organs shows the various stages of the process and establishes beyond doubt a true histogeny, though it has not been possible to demonstrate the details of mitosis in the proliferating cells. This may be due in part to lack of just those refinements of technique necessary to bring out these features. Some of the tissues were fixed by means of Flemming's solution, some by corrosive-acetic acid, and still others in 10 per cent. formol in water. I have not been able to distinguish that there was any appreciable advantage in the one over the others, the formalin seeming to afford equally good fixation and preservation. Heidenhain's iron haematoxylin and an aqueous solution of haematein both afforded fairly good differentiation, though they failed as to the nervous tissues, a result which was not unexpected.

In Figure 2 is shown a longitudinal section of a regenerated rhopalium at a comparatively early stage, when first distinguishable as a minute papilla. In an earlier part of the paper I have referred to its early appearance as having the character of a solid bud from the upper angle of the notch made in the process of excising the organ. From an examination of this figure, which is among the earliest stages I have been able to satisfactorily section, it would seem that in its origin it probably follows the usual process of the regeneration or development of such organs in the coelenterates, namely, that of budding, involving both ectoderm and entoderm. As shown in the figure, there is here a typical outgrowth from the distal end of the radial canal and, as also mentioned in another connection, it was easy to demonstrate at about this stage of development in the living medusa an active circulation in the bud. The cells of the ectoderm at this stage are of approximately uniform size over the entire organ, and the same is also the case with the cells of the entoderm. There

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seems also to be present the middle lamella, though less sharply defined than at a somewhat later period. There appears to be a rapid proliferation of the cells of the entoderm near the terminal portion where they form a mass as shown in the figure, though, as mentioned above, it was not possible to distinguish evidence of mitosis.

It is interesting to note at even this early stage the incipient phases in the regeneration of the sensory areas (Fig. 2, *s. e.*) just above and below the rhopalium. The regeneration of the hood is also shown at *h.*

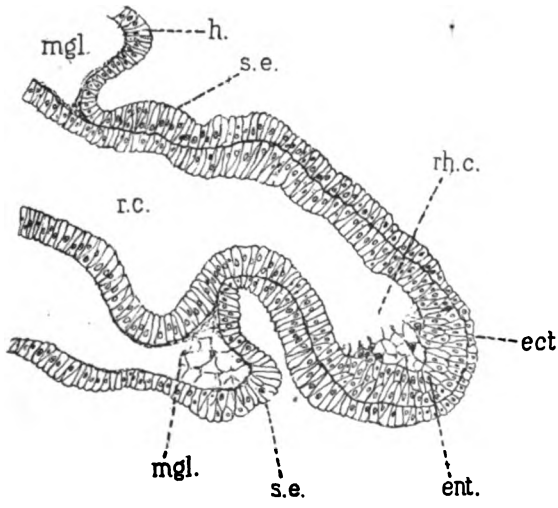

FIG. 5.

Section of regenerating rhopalium. rh. c., rhopalial canal; other letters as in Fig. 2.

In Figure 5 is shown a section taken in the same plane as the former, but at a somewhat later stage of development. The rhopalium has apparently attained nearly full size, but lacking as yet any development of otoliths, though in the network shown at *rh. c.* there is apparently evidence of a differentiation preparatory thereto. There is also shown here the thinning out of the ectoderm of the distal portion of the organ as seen at *ect.*

The sensory areas and epithelium above and below are here seen to have acquired almost their typical form and character as

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shown at *s. e.* The hood is also shown at *h,* not having apparently kept pace with the growth of the other organs. Here as before the direct connection of the radial canal with the rhopalium is quite broad and characteristic. The middle lamella, or mesogloea, is shown at *mgl.* above and below, in the latter elements of a loose network being traceable, with the embedded cells, which can also be found indefinitely scattered throughout the jelly.

In Figure 6 we have a section through an almost mature rhopalium, taken in the same plane as the others, only the organ

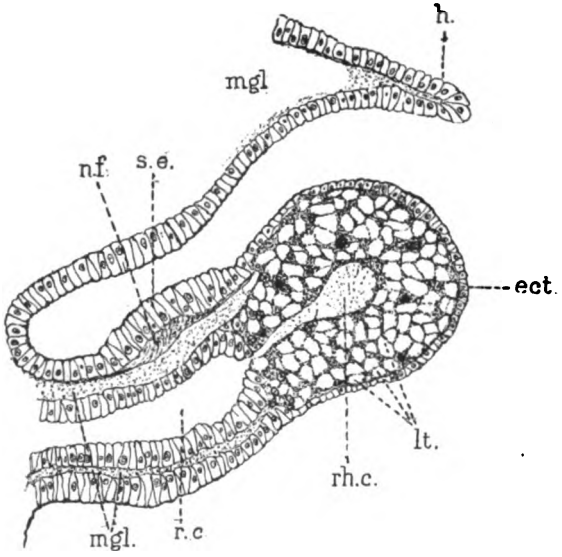

FIG. 6.

Section of regenerated rhopalium, approaching maturity. lt, lithocysts; nf, nerve fibers; other letters as in Figs. 2 and 5.

itself with the terminus of the hood being shown. The ectoderm has become practically uniform over the entire distal portion of the organ, but as it approaches the base of the area of the lithocysts, shown at *lt.* and generally throughout the entire distal part, it becomes columnar. At *s. e.* it forms a definitely arched portion, the sensory epithelium, beneath which at *n. f.* is the so-called nerve fiber area of the nerve center of this region. While it is quite possible to distinguish a more or less fibrous character as

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shown in the figure, it has not been possible to trace these fibers into any cellular plexus, or ganglion, such as has been claimed to exist here. Since however my observations in the present instance have been almost entirely restricted to phases of regeneration, it will not be pertinent to discuss the question farther.

As will be seen there is still a continuous connection between the cavity of the distal portion of the organ and the radial canal. This connection Hesse, '95, has shown in figures of normal organs in maturity, but in the present examinations I have found it when fully regenerated to become entirely solid throughout the lithocyst region, the radial canal ending abruptly at its basal end, which is shown almost closed in the figure under consideration.

In the rhopalial cavity, *rh. c.,* which at this time is nearly spherical, there is present a radiating network of delicate fibers, poorly shown in the figure, which seem to diverge from a point on the lower surface and extend entirely across the cavity apparently attaching to the opposite wall. I should consider these fibers of the same nature as those shown in Figure 5 near the terminus of the line *rh. c.*. Though it has not been possible to critically trace the details of the process it seems entirely probable that the entodermic epithelium of this region becomes gradually differentiated into fibers which form the intricate network within which the lithocysts are later deposited. Within this network may be found during the various stages of development the gradual metamorphosis of this entodermic cell mass, the nuclei of the cells often remaining as permanent elements of the organ. Some of the more prominent of these are shown in the figure, and phases of the metamorphosis may be detected near the narrow slit-like canal, just beyond the terminus of the radial canal.

Within the network may also be traced the deposition of the pigment characteristic of the organ.

Concerning the histology of the regenerated oral and gastric organs it has not seemed essential to make special inquiry, since in what has already been shown in connection with the more highly differentiated tissues of the marginal organs it would seem that no serious doubt can remain as to normal histogenic pro-

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cesses probably occurring throughout every regenerating organ in this medusa.

It was pointed out in connection with the description of certain experiments that both in form and in function we have among the Scyphomedusae a regenerative capacity extending to the most highly specialized organs. In the subsequent account of the histology of the regenerated organs it has been shown that the process is a perfectly normal and characteristic one, conforming in apparently every detail to the course of development of the embryonic history of the several organs.

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Syracuse University,
The Zoölogical Laboratory,
January 20, 1904.

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NOTES ON A HITHERTO UNDESCRIBED HYDROID FROM LONG ISLAND SOUND.

CHAS. W. HARGITT.[1]

Early during the present year I received from Dr. Henry R. Linville, of New York City, a small collection of hydroids for examination, among which was found what clearly appears to be a new species. The specimens were collected by Dr. Linville during the previous August near East Marion, Long Island. In a letter to the writer he describes the habitat as follows:

"The species was found growing on rocks and piles under 'Milldam Bridge,' west of Shelter Island. The bridge spans a narrow creek which connects the Bay with a shallow salt-water pond."

In size and general features the hydroid resembles very much *Syncoryne mirabilis*, and was at first thought to be that species. A more critical examination showed this to be more than doubtful, as the number of tentacles, character of the hydranth, and above all the character of gonophores, all indicated otherwise, and a review of the accessible literature failed to afford any clue to the identity of the species, though it seems clearly a member of the genus above named as will be seen from the sketch.

The medusa-buds are relatively large and grow in clusters of from two to four upon the body of the hydranths usually close among the tentacles. I regret that no free medusæ were obtained and that only an incomplete description of this organism can be made. As will be observed from the figure the medusæ present the somewhat unusual condition of having at this stage two of the tentacles quite well developed, though short and thick, at opposite positions on the margin of the umbrella. The intermediate pair can hardly be distinguished, being mere buds, and it is impossible to say whether they later develop or not; indeed, it remains doubtful as to the later phases of development of almost all the medusoid organs, though there can hardly be serious doubt as to the ultimate freedom of the medusæ, as there were

[1] Contributions from the Zoölogical Laboratory, Syracuse University.

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no signs of the development of gonads, a condition almost certain to have been found in case the gonophores remain sessile.

The following diagnostic characters will serve to distinguish the species, at least so far as the hydroid phase is concerned, for which the name *Syncoryne linvillei* is proposed:

Trophosome. — Colony growing in tufts, sparingly branched, to a height of 15 to 30 mm., and with the same general aspects as characterize *S. mirabilis* Ag. Hydranths vasiform, with cone-

Portion of colony of *Syncoryne linvillei* about three times natural size.

shaped proboscis; tentacles definitely capitate, from 15 to 30 in number, and variously distributed over the proximal third of the hydranth body. Perisarc much as in *S. mirabilis*, plain, or with only the slightest trace of any annulation, ending somewhat abruptly below the base of hydranths, proximal or basal portion dense or brownish in color. Hydrorhiza more or less reticulated, forming a loose network over the substratum. In all the type specimens both hydrorhiza and hydrocaulus were enmeshed in a dense sponge-like mass, though its exact nature is doubtful.

Gonosome. — Medusa buds borne on body of hydranth, usually in small clusters among the bases of the tentacles and supported by a single peduncle, the terminal specimen always maturing

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first. In the type specimens no free medusæ were present, though there were numerous buds approaching full development. The umbrella is distinctly bell-shaped, with four radial canals; tentacles unequally developed, two on opposite sides large and club-shaped, the intermediate pair small and bud-like. No ocelli distinguishable.

Colors. — Formalin material pale yellowish, hydranths somewhat brownish; gonophores reddish brown or pink.

Habitat. — Growing in tufts upon small rock fragments, bits of bark or on shelly concretions; the bases of stems tangled or enmeshed in sponge-like masses, the tufts and spicules of which give to the colonies a rough and bristly aspect.

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The Early Development of Eudendrium.

By

Chas. W. Hargitt,

Syracuse University, Syracuse, N. Y.

With plates 14—16.

While not a little has been done by various observers on the general character and life history of the *Eudendridae*, including more or less critical investigations upon the origin of the sex-cells, very little has been done, so far as I am aware, in the details of development of any of the species of the genus *Eudendrium*.

My own interest in the group began several years ago in relation to the origin of the sex-cells, brief reports upon which have appeared elsewhere and will be further referred to in another connection. While engaged on this phase of the subject I became impressed by some apparent anomalies in the ontogeny of one of the species. But for various reasons its critical study has until recently been delayed.

It is the purpose of the present paper to present in some details the origin of the ova and early development of *Eudendrium ramosum*, including briefer accounts of two or three other species.

While most of the material was collected and considerable of the work done at the Marine Biological Laboratory, Woods Holl, it has been carried to its present state of completion during the occupancy of the Smithsonian Table at the Naples Zoological Station, for the opportunity and courtesy of which I am under obligations to the Secretary of the Smithsonian Institution, Mr. S. P. Langley, LL. D.

Material and Methods.

Most of the material was obtained at Woods Holl, that of the species of *E. racemosum* and *E. capillare*, at Naples. The collections

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were made at varying seasons, chiefly June, July and August at Woods Holl and during May and June at Naples, and in each case during at least two years, so that it may be safely assumed to represent average conditions of growth, maturity, etc. The preservation of material was under direct personal observation and in all cases upon perfectly fresh specimens and by a wide range of reagents, including hot sea-water, the various forms of Mercuric chloride, with and without acetic acid, and used both hot and cold; the various picric acid preparations, FLEMMING's solutions, weak and strong, HERMANN's solution, PERENYI's solution. The various preparations of formol, pure alcohol alone and with such reagents as formol and Mercuric chlorid in solution. While good fixation was obtained by both HERMANN's and FLEMMING's solutions, subsequent staining proved difficult and unsatisfactory. Upon the whole the best results were obtained from fixation with a strong alcoholic solution of Mercuric chloride. The several picric acid formulae have proved unsatisfactory in the fixation of coelenterate material, whether of hydroids, medusae, or their ova. Depending in my earlier collections upon the general repute of this reagent they were later found, greatly to my surprise and regret, to be almost worthless. PERENYI's solution gave only fairly satisfactory fixation. I have found in some cases excellent results from a 10% solution of formol in sea-water, tho prepations in this medium were not constant, nor did stronger solutions prove any more satisfactory. In some cases a mixture of equal volumes of absolute alcohol and glacial acetic acid gave good results.

Origin of the Sex-cells.

Since the announcement of CAVOLINI (1785) that Hydroids produced eggs, although what he at that time supposed to be such were probably larvae, the subject of sex-cells has been a matter of extended interest and research. The researches of EHRENBERG, SIEBOLD, SARS, STEENSTRUP and DUJARDIN, established the fact and law of the "alternation of generations".

VAN BENEDEN (1843) seems to have earliest directed attention specifically to the question of the origin of the sex-cells in work done on Hydroids and announced that the eggs originate in the cells of the endoderm, while spermatozoa arise in the ectoderm. He proposed this as probably true throughout the animal kingdom, an illustration of hasty generalization from extremely limited data, and with the futile results usual in such cases.

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The brothers HERTWIG (1878) following up their researches upon the histology of the medusae, with investigations upon the origin of the sex-cells, announced that in medusae they were to be found in the epithelium of the sub-umbrella and from a somewhat limited range of comparisons announced the conclusion that among all Hydromedusae the sex-cells arise from the ectoderm and further that among the Scyphmedusae the sex-cells have their origin among the cells of the entoderm, a condition also true for the Anthozoa which had come under observation.

The extended researches of WEISMANN (1880, 1881, 1883, 1885) culminating in his classical monograph, 1883, limited almost exclusively to the Hydromedusae, marked apparently the climax of interest so far as it related to this phase of the subject.

However, investigations have continued with more or less interest up to the present time and not a little revision of WEISMANN'S conclusions has been necessary due in part to the unsatisfactory and inconclusive methods which he had employed in his earlier researches. It should be noted that the work of WEISMANN had reference, not only to question of fact as to the simple matter of the origin of these cells but as to its bearing upon such problems as the relative rank of the hydroid and medusa, and further concerning its value as a means of classification.

WEISMANN has described in considerable detail the origin of the sexual cells in *Eudendrium*, as has also KLEINENBERG, the latter observer contending for the origin of ova wholly from the ectoderm in contradiction of the former, who allows the possibility in *E. capillare* of an entodermal origin. Some reference to this apparent contradiction will be made in another connection.

In a previous contribution (1889), I was led to conclude that in the American species of *E. ramosum* the ova have their origin in the entoderm. The somewhat emphatic contention of KLEINENBERG (1881) to the contrary which later came to my attention, as well as later observations upon these and other hydroids by others led me to a more critical reexamination of my earlier work and its extension to several other species of *Eudendrium*, namely, *E. racemosum* CAVOLINI, *E. dispar* AGASSIZ, and a very small species found at Woods Holl, probably *E. tenue* AG., also *E. capillare*, and in brief with the following conclusions:

1) In *E. racemosum* CAVOLINI, ova are undoubtedly found in both ectoderm and entoderm, but the larger were invariably in the ecto-

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derm, and the smaller as invariably in the entoderm, as shown in Fig. 1. The inference would seem to be warranted that their origin is in the latter tissue from which they migrate into the ectoderm through which they further migrate to the gonophores. I have not been able to certainly demonstrate the actual passage of ova from the one tissue to the other as WEISMANN seems to have done, or as BRAUER has figured in the case of *Tubularia mesembryanthemum*, though do not question it as a fact.

2) In *E. dispar* AG. there were found identical conditions to those described for *E. racemosum*. Ova abound in both tissues, but the larger and more mature are found invariably in the ectoderm. In the region of the hydranth ova were found, as in other portions of the body, but were here always approaching maturity.

In a few cases a condition which had every appearance of the passage of the egg directly through the supporting lamella was observed in the region near the gonophores tho not elsewhere. Fig. 2 represents a camera sketch of a long section of this hydroid showing the relative size and position of ova.

3) In *E. tenue* AG. a distinctively different condition was found. This is a very minute hydroid and correspondingly difficult to study. Only transverse sections were made, a camera sketch of one of which is shown in Fig. 3. In no case were ova found in other than the entoderm, as shown in the figure. The specimens were taken during the height of the breeding season and with sexual products in all stages of development.

4) *E. ramosum*. As intimated above I have taken occasion to carefully review my earlier work on this species with the result that the later observations confirmed in every detail those originally made. Fig. 4 presents an average condition found in this hydroid during its reproductive season.

5) An examination of *Eudendrium capillare* ALDER confirms in almost every detail WEISMANN'S conclusions and are in such perfect accord with the conditions found in *E. tenue* as to further suggest the long suspected identity of these species. I should state that in a single case among hundreds of sections of this hydroid there was found an egg in the ectoderm in the region of a gonophore, which was approaching the state of maturity usually attained about this time, which suggests the probability that it had passed into the ectoderm in this region in approaching the gonophore during the process of migration thitherward.

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Reference was made in a preceding paragraph to the apparently contradictory observations of KLEINENBERG and WEISMANN. It seems to me this may be rather apparent then real. In the first place several species of *Eudendrium* so closely resemble each other at certain stages of development as to render it easy to mistake the one for the other.

In the next place, there seems to have been assumed the inference that the species of a given genus should have the same mode of origin the sex-cells. This is clearly an unwarranted assumption as the foregoing observations, as well as many others by various observers, abundantly demonstrate.

KLEINENBERG's suggestion that the differences may be due in some measure to seasonal conditions to which sexual processes may be more or less responsive seems to be wholly inadequate as an explanation. In the cases above cited, which were all obtained under substantially identical circumstances and at approximately the same season, it can, of course, have no significance. It would seem more probable that there are undoubted variations in the same species arising from intrinsic conditions of nutrition, or similar physiological changes, and granted the fact of migration of these cells their response to these changes is only what might be naturally expected. Moreover, it is not altogether improbable that such conditions of metabolism might measureably influence the origin of the sexual products as well as their development.

Growth of the egg in the *Eudendridae*. It is no longer matter of doubt that the ova arise in these hydroids by differentiation of cells of either one or both the entoderm and ectoderm. It may be in the region of the hydranth or lower in the coenosarc of the stem. When first distinguishable they are slightly larger than the ordinary cells of the surrounding tissue, and differ also in shape, being generally ovoid or spherical and with comparatively conspicuous nuclei. Their form and size generally suffice to distinguish them from the glandular cells often distinguishable in these hydroids, though in their staining reactions they closely resemble the latter. Growth at this period would seem to take place in situ, through the direct nutritive activity of the surrounding tissue cells. In texture the cytoplasm is homogeneous, finely granular and with less staining avidity than the nucleus, which from the first is large, with a prominent nucleolus and with ready affinity for stains of almost any of the commoner sorts. As growth continues the ova become more or less amoeboid, migrating

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toward the gonophore region where they seem to aggregate in considerable numbers, the presence of which may act as a stimulus from which results the formation of the gonophore. These organs may arise singly or several may arise in close connection forming clusters, into which the ova readily migrate. Almost immediately following their final position in the gonad a change becomes apparent in the character of the cytoplasm of the egg. It loses its finely granular and homogeneous character and acquires a store of deutoplasm which assumes the form and special characters of yolk spheres.

These first appear about the periphery of the egg and are of large size. As they accumulate by the constant formation of new spheres within the cytoplasm of this region they become massed toward the center of the egg, thus crowding the cytoplasm toward the surface. Indeed it may not be improbable that during this period there may exist more or less active cytoplasmic movements which are effective in the final disposition of the egg contents. This is further suggested by the gradual change of position by the nucleus in the later phases of growth and approaching maturation, as shown in Figs. 5—7. It should not be overlooked however, that the nucleus may possess intrinsic powers of migration in these eggs, as strongly implied in the pseudopodial processes found in many cases, as shown in Fig. 6.

That the yolk spheres arise directly through the cytoplasmic activity of the egg there can hardly be any doubt, tho their substance comes indirectly from the metabolism of the entodermal layers of the gonophores, which with the growth of the egg is gradually reduced in mass till its cellular character is almost or wholly lost.

Various phases in this line of metabolism are easily traced in hydroids with fixed gonophores but it is unnecessary to submit details in this connection.

As these yolk masses increase a change takes place in the character and position of the nucleus. It no longer occupies the center of the egg but migrates toward the periphery, or it may be that the increase of food spheres which aggregate in the center of the egg forces the nucleus into an eccentric position, along with the cytoplasm which likewise becomes more or less peripherally disposed. Change of position is not however the most important which overtakes the nucleus during this period of the growth of the egg. Changes of texture of the nucleus become more or less apparent both in living eggs and in the reactions shown by staining agents.

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The nucleoplasm becomes more coarsely granular than before, the nucleolus disintegrates, the nuclear membrane disappears and the nuclear contents seem to dissolve and be distributed throughout the cytoplasm. Whether certain of these changes may not be associated with fertilization may well be probable, tho just how far or in what way I have not been able to determine. The whole problem of fertilization among hydroids with closed gonads similar to those of *Eudendrium* is involved in uncertainty. But however this may be there seems hardly any doubt as to the fact of the dissolution of the nucleus about the time of the full growth of the ovum. That it bears some relation to the fact of fertilization is further suggested by the signs of cleavage which follow soon after these changes have occurred (cf. Figs. 5 and 6).

Coincident with this rapid accumulation of deutoplasm by the ova of *Eudendrium* is the decrease in the size and mass of the entoderm of the surrounding walls of the gonad, a fact which is probably to be interpreted as due to the direct contribution of these tissues to the nutritive metabolism involved. When fully mature the ova of these hydroids is of an orange color and more or less opaque, rendering any critical study of internal changes on the living egg at this time quite difficult or impossible. The abundance of the yolk substance is likewise a barrier of great difficulty to satisfactory fixation and cutting of the eggs as well as subsequent staining and clearing.

Reference to fertilization has already been made incidentally, and little can be added further. So far as I am aware nothing specific is known as to this phenomenon in such hydroids as *Eudendrium*, where the ova are enclosed within sporosacs or gonads with thick walls which at no time have direct communication with the outer or inner surfaces of the animal. In the case of *Hydra* which has been supposed to have its eggs fertilized by sperms penetrating the wall of the ectoderm, this might be possible, where only a thin tissue separated; but with some forms, like the one under consideration, or with *Campanularia* where a chitinous gonangium must prove a serious impediment to such access, the case is quite different. Examination of the living hydroids repeatedly for some evidence as to the process has as often failed absolutely to afford any satisfactive clue.

Similar uncertainty as to the phenomena of maturation of the egg prevails. So far as the *Eudendridae* are concerned nothing is known as to the superficial phenomena, e. g. polar bodies. In many hundreds of eggs sectioned no trace of polar cells could with cer-

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tainty be recognized. And it is hardly necessary to suggest that its observation in living eggs is all but impossible. The same is to a certain extent the case with other hydroids of similar character. Even with forms in which the eggs are set free the phenomenon is rarely conspicuous. As I have elsewhere noted, in only a few cases have I identified these cells with certainty in *Pennaria* ova, which are freely discharged before fertilization occurs.

Similar difficulty exists in most cases in reference to the internal features of maturation. This has been already hinted at in a previous paragraph. With *Eudendrium* it is particularly difficult owing the massing of yolk spheres of very opaque nature in the egg, and to the distortion, or apparent disintegration of the nucleus during the growth of the egg. HICKSON has found a similar condition in the growth of the egg of *Distichopora*. Indeed, many of HICKSON's figures would serve to portray with fair accuracy the prevalent condition found in the hydroid just cited. The migration of the nucleus to the periphery of the egg and the amoeboid aspect of the nucleus during this time and its subsequent apparent disintegration and dissipation through the cytoplasm of the egg have seemed to me to be associated with maturation, rather than with fertilization as HICKSON has suggested.

COE (1898), has shown that in *Cerebratulus* maturation of the egg is accompanied with the discharge of no inconsiderable portion of the chromatin and its disintegration and distribution through the egg cytoplasm. Something similar to this apparently occurs in *Eudendrium* and several other hydroids and hydromedusae, perhaps in a considerable number. In these cases however it is not necessary to assume that the chromatin thus dissipated through the cytoplasm finds no further use in nuclear activity, as COE has suggested concerning *Cerebratulus*. Its dissipation is only a phase in the maturation of the egg associated with the dissolution of the nucleus and its probable distribution throughout the cytoplasm.

So far as I have been able to follow the course of maturation phenomena in *Eudendrium* they are substantially as follows:

Following the migration of the nucleus toward the periphery of the egg, as previously intimated, there is complete dissolution of the nuclear membrane, and the total disappearance of the nucleolus, whether as a whole, or by dissolution and dissipation I have not been able to demonstrate certainly, but failure to trace its discharge as a distinct body leads me to infer its fragmentation and gradual dissipation. Fig. 7 shows a final condition of the nucleus, apparently

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devoid of all trace of nucleolar substance with but slight trace of chromatin granules. Very soon after this phase the nucleus becomes more and more transparent, completely losing its chromophilous properties, and only rarely being distinguishable in even the thinnest sections.

As intimated above no trace of polar bodies has been found, and I am constrained to the opinion that following their formation soon after the dissolution of the nuclear membrane they are probably resorbed by the cytoplasm, as HARM (1902), has shown in the case of *Clava*, and as in other well known cases.

Early Development.

Observations made upon living material of several species, more particulary *E. ramosum* and *E. capillare*, showed phases of cleavage comparable with Figs. 8—13, drawn under the camera from life of *E. ramosum*. It was indeed possible to follow in some cases the progress of the cleavage, tho not with any considerable detail owing to the opacity of the eggs and the difficulty of orientation, since not only the individual gonophore but a considerable portion of the hydroid colony seemed necessary in order to maintain normal conditions even for a short time. By a comparison of several specimens it was not difficult however to recognize that in surface aspects they bore every semblance of ordinary cleavage phenomena. Upon fixation, however, by whatever reagent employed every trace of cleavage disappeared, and subsequent sectioning failed likewise to afford any internal evidences of definite blastomers or other cleavage characters. It was only after repeated experimentation with every available reagent that I was forced to the conclusion that in all probabilities the surface phenomena were not true cytoplasmic, but yolk-cleavages, comparable with those of Crustacea and Insecta. That they are not however wholly independent of cytoplasmic, or nuclear activity seems more or less evident since sections show the presence of nuclei in various phases of activity, mitotic figures being demonstrable and resting nuclei quite common. These were however, for the most part wholly independent of the yolk, the latter being more or less evident in early stages as pyramidal masses, somewhat similar to yolk-pyramids in Crustacea, as shown in Fig. 15. Again the irregular distribution of nuclei suggests conditions more or less common in insect cleavage. It would seem therefore that we have in the heavily yolk-ladened eggs of the *Eudendridae* cleavage features comparable in many respects with those found among similar eggs in other groups, more evident

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and general in Crustacea and Insecta, tho not lacking in others, e. g. Myriopoda and Arachnida, where similar conditions are found.

Briefly summarized the phenomena of early development appear to be somewhat as follows: Upon the maturation and fertilization of the egg, as previously described, and following the apparent dissipation, or perhaps fragmentation of the nucleus, its early reorganization into one or more nuclear centers constitutes the initial impulse of development. With the massing of deutoplasm in the center of the egg the cytoplasm, as already noticed, has been forced into a peripheral layer, tho with something of a reticular network throughout the entire egg. In the nuclear activity and division which now takes place the cytoplasm is but slightly involved, or at any rate no specific divisions occur within its mass giving rise to independent cells, tho cytoplasmic condensations occur in close relation with each nuclear center, as shown in Figs. 24 and 25. Under the stimulus of these associated activities the deutoplasm within the cytoplasmic reticulum becomes also involved and a definite cleavage occurs in a more or less orderly fashion till the entire mass becomes divided in a series of cell-like bodies, or yolk-balls, composed of the primary yolk-spheres, which are of varying size and shape. Within some of these yolk-balls are found resting, nuclear-like bodies, perhaps true nuclei, but in others no such centers are demonstrable. The nuclear character of these is furthermore strongly suggested in that, as will be farther noticed later, they appear to become centres of active metabolism, and indeed of nuclear proliferation.

These superficial aspects of cleavage never appear to go beyond that shown in Fig. 13, including perhaps from thirty, or at most fifty such cells. Internally however the case seems quite otherwise. Following the yolk cleavages and perhaps including also to some extent the cytoplasm as well, a condition obtains wherein the cleavages are for the most part limited to the nuclei, which seem to multiply for a time with great rapidity till a superficial layer is formed about the entire surface of the egg. This layer is not however uniform over the surface till a comparatively late stage. It seems to begin usually at a given area, presumably richer in protoplasm and to gradually extend to the remoter parts. Coincident with the extension of these nuclear bodies, for there are not as yet any evidences of cell boundaries, the egg at this time being in fact a syncytium, and continuing such during the entire period of egg development, over the surface, there has also been an extension between the yolk balls,

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among which there has apparently been from the first a coarse, cytoplasmic reticulum as shown in Fig. 21. At certain regions of this reticulum this proliferation of nuclei seems to be more active than elsewhere, as shown in Figs. 17—20 where they form nest-like clusters within which later occurs definite cell organization. Such a nest is shown in Fig. 22.

An examination and comparison of several of the figures cited would seem to indicate something like a migration of the nuclei from place to place, where following later multiplication, they become centers of more or less active metabolism, involving the disintegration of the yolk-masses and their direct transformation into a proteid substance more easily available by the growing cells. By means of various differential staining it was possible to trace in more or less detail the progress of this metabolism which beginning with the peripheral yolk spheres gradually extended toward the center till the entire mass was disintegrated and liquefied. At first these yolk spheres always took a very deep stain with any of the hematoxylins used, with HEIDENHAIN's they became densely black. Following their disintegration the staining properties became much less intense, or wholly different.

Amitosis. It should be remarked in this connection that while in the very early cleavage phases coincident with the yolk cleavage and probably in some way associated therewith as shown in Figs. 24 and 25 there were abundant mitoses, yet in the later stages, particularly those involving the apparent migration of nuclei and their multiplication within the yolk-masses they seem to be conspicuously lacking. The syncytial character of the early embryo has already been referred to. During this period, specially in its earlier history, the nuclei differed greatly both in size and shape. Some were many times larger than others, while adjacent nuclear nests often showed indications of having resulted from the amitotic division of a larger nucleus. Again, many nuclei in their elongated and dum-bell shapes also suggested strongly phases in amitosis, as shown in Fig. 23 *a*, *b*. Another interesting and suggestive fact in this connection is the occurrence of string-like series of nuclei along, or within, threads or strands of the cytoplasmic reticulum, as shown in Fig. 21.

It need hardly be stated that this condition was found under the most varied methods of fixation and staining, both from preparations stained in toto and on the slide, and by subsequent decolorization and the application of an entirely different stain.

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While the mere absence of mitotic figures at a certain stage in the development of the egg embryo may not warrant a dogmatic assertion as to amitosis, it would seem that the several series of evidences above cited and the easy demonstration of mitosis at a preceding stage strongly suggest its probability.

But other cases are not wanting which more or less warrant the conclusion that amitosis as a cytologic phenomenon is not restricted to degenerating tissues or cells, tho perhaps better known in such connection, nor yet to the fairly well established facts of spermatogenesis. There seems no longer reason to doubt its occurrence at least in certain phases, in early embryonic development.

The convincing researches of MEVES (1898) and others in spermatogenesis would seem to leave little doubt that the amitosis admittedly occurring here involve the spermatogonia no less than the nutritive, or supporting cells (SERTOLI cells).

Similar observations are not lacking concerning amitosis in oogenesis and in the early history of the embryo. EISIG's (1899) recent paper on the development of the "Capitelliden" affords substantial evidences of amitosis in several stages of early development in these annelids, as did the earlier paper of WISTINGHAUSEN (1891), on the development of *Nereis*.

MEISENHEIMER's recent paper 1902, on the development of the "Pantopoden" shows a condition following the cleavage similar in many respects to that found in *Eudendrium*, in which following a partial invagination resembling gastrulation there is formed apparently by amitotic cell division an "inner cell-mass" from which there are later differentiated the entodermal and mesodermal tissues and organs.

HEATHCOTE (1886) in describing the development of *Julus* gives phases of nuclear origin and migration which likewise seem to have points in common with *Eudendrium*, the nucleus becoming indistinct and apparently dividing amitotically.

HERRICK (1892, development of the Macroura, p. 397) has described a condition in the development of *Alpheus minus*, which likewise has features comparable with those in *Eudendrium*. To quote briefly in his own words. "Division appears to be direct and irregular. At a somewhat later stage the phenomena of the most interest occur. Each product of the first nucleus has developed a swarm of nuclear bodies which seem to arise by fragmentation. These bodies take the form of spherical nuclei in clear masses of protoplasm", and later constitute a considerable portion of the entire egg.

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Formation of the Ectoderm.

Attention has already been directed to the migration of nuclei from the midst of the yolk spheres toward the periphery of the egg. Here an indefinitely reticulated ectosarcal layer of cytoplasm early appears and within this layer the nuclei find their places, increasing in numbers, both by the process of migration as well as by free multiplication, till a fairly well distributed series is formed over the entire surface, as shown in Figs. 18—20, tho there are as yet no signs of distinctive cell boundaries or cell individuality. As will be noted from an inspection of the figures just cited, as well as from several others of slightly later stages, this peripheral layer does not at this time constitute a definite ectoderm, lacking the histological characters of a distinctive tissue, as already intimated, or at any rate the distinctive features of true ectoderm. That it does constitute an ectodermal border, or probably, an ectodermal anlage, does not seem in the least doubtful, and may therefore justify the designation of primitive ectoderm. In view of the more or less marked changes which occur in its development the latter might seem upon the whole the more appropriate designation.

It should be noted in this connection that the protoplasm of this layer is in some cases rather granular, in others more reticular, and often in both conspicuously vacuolated. This last feature is more or less evident throughout the entire course of development, becoming more so as the ectoderm assumes final form.

As the nuclei increase in number they come to form a more or less symmetrical series, giving to the layer the aspects of a distinct tissue though as yet without evident cell-boundaries.

In the earlier phases of development the nuclei, as well as the cytoplasm, of the primitive ectoderm differ considerably in size as well as in number and the thickness of the cytoplasmic border. This will be evident from an inspection of the figures just cited. With the progress of development these differences diminish but do not wholly disappear, even in the mature embryo or free-swimming planula, as will be further shown later on, nor is the development of the ectoderm uniform over the entire embryo. It may often be seen to be fairly well differentiated at one pole, usually that destined to become aboral, while it remains for some time indefinite at the opposite pole.

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With the approach toward differentiation there occurs a more or less evident cellular change, the cytoplasm concentrating about the nuclei, in cubical blocks, but still without the formation of a cell membrane, which does not appear till sometime later.

Glandular cells. About this stage in the history of the ectoderm there appear certain cellular elements which are destined to form a conspicuous feature of the later development. On the inner border of the now fairly differentiated ectoderm appear large, oval, more or less vacuolated cells with usually prominent nuclei, and with their long axes parallel with the surface of the embryo. Their cellular cytoplasm is definitely reticular with a more or less granular mass about the nucleus, and in many cases the entire cell highly vacuolated. The fact that these vacuoles remain under the varied phases of staining show that they are probably filled during life with watery cell-sap, or thin mucous. They are in all probability glandular cells, supplying possibly during later embryonic development a digestive ferment which contributes toward the further reduction of the yolk masses and their transformation into available proteids. With the growth of the embryo their position tends toward the surface and with the escape of the embryo they have assumed a position more nearly parallel with that of the ordinary ectoderm cells, or with their long axes at right angles to the surface. This would naturally comport with their now changed function as mucous cells which supply the abundant mucous or slime, so characteristic of planulae of nearly all hydromedusae, and particularly of those of the *Eudendridae*. These larvae, while quite free, and swimming for a time near the bottom of the aquaria, always leave a trail of slime, which sometimes in small dishes becomes so abundant as to more or less tangle or enmesh them, and also to render difficult their easy separation individually for transfer or for fixation and preservation.

Figs. 22—30 drawn by aid of the camera will show the several forms and aspects of these cells at various stages of development.

With the release of the planula from the gonophore and during its free-swimming life these cells become relatively more abundant in the aboral region, which is what might be naturally expected as one considers the secretory function necessary during the later larval life in the fixation of the young polyp and the secretion of the perisarc about its base. I have not undertaken to follow their history during the later development of the hydranth, nor of course, in the mature

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hydroid. The extended investigations of this last feature by WEISMANN (1883), JICKELI (1883) and others render such unnecessary.

Interstitial cells. These cells make their appearance about the time of the distinct differentiation of the true ectoderm as very minute nuclei usually located among the basal, or interior ends of the elongated and larger ectoderm cells. They continue small, and of a more or less oval or pyriform shape, with a tendency to form clusters or nests in a given place. Their later history following the liberation of the planula I have not undertaken to follow.

Entoderm Formation.

From the preceding account of the origin and development of the ectoderm it will be at once apparent that the formation of the entoderm must be somewhat different from the process usually involved in this phase of development. Whether in Coelenterates or animals of more complex organization. Certain it is that neither invagination, delamination nor multipolar immigration, is involved in the development of *Eudendrium*. With the proliferation of cells centrally and the migration and further proliferation of certain of these to form the ectoderm, and with the continued proliferation of others till a central undifferentiated mass of nuclei forming a syncytium, occurs nothing comparable with the phenomena involved in the above named processes can be recognized. Furthermore, it is quite evident that up to the time of the escape of the larva no definite entoderm is yet formed at all (cf. Figs. 26—28). This condition continues for some time during the free-swimming life of the larva and it is only with the approach toward metamorphism into the hydranth, that a final differentiation of a definite entoderm takes place, involving only a portion of the internal, syncytial mass, while remnants of yolk, nuclei and cytoplasm are gradually disintegrated, digested and serve as food for the young organism during its final larval life.

I have shown that a similar condition obtains in the development of *Pennaria* (1900). ALLEN (1900) in describing the development of *Tubullaria crocea*, while giving no details as to entoderm development, refers it to HICKSON's type of "precocious delamination". A careful review of her figures and a later study of her preparations convinces me that it is extremely doubtful whether any delamination in the true sense occurs here. Indeed I think the same is also true conerning BRAUER's account of *T. mesembryanthemum* (1891).

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Life History of the Planula.

In general the life hystory of the *Eudendridae* is quite similar to that of other Hydromedusae, only those features which are more or less distinctive will be noticed. In the general shape there is nothing peculiar. When first liberated they are elongate pyriform, becoming much more slender after a few hours or a day of the free-swimming condition. The planula of *Eudendrium* is from the first abundantly ciliated, much more so than that of *Pennaria* or *Clava*. By a curious oversight, which I desire to correct in this connection, the planula of *Eudendrium* which was under observation at the same time with those of *Pennaria*, of both of which drawings were made, that of the former was inserted in the paper on the latter, fig. 7, tab. 1, to which reference may be made instead of its reinsertion in the present.

The free swimming period is comparatively brief, rarely exceeding two or three days, usually less. At first the movements are relatively rapid, particularly when compared with those of *Pennaria* which are quite slow. A point of considerable interest in this connection is that of the heliotropism of the planulae. This is specially marked during the first few hours following their escape from the gonophores. Their liberation seems to be chiefly during early midday from colonies brought into the laboratory during the morning. In those collected during the late afternoon very few planulae were set free. When liberated from large colonies at the height of the breeding season their numbers were large and they promptly swam directly toward the strongest light with great uniformity. By interposing a dark screen between this source of light and allowing another from the opposite side to operate upon the aquarium, there was an almost instantaneous response, the entire number almost without exception facing directly about, like a body of soldiers at command, and moving without deviation in the opposite direction, that is, toward the second source of light. The experiments were so varied and repeated as to leave no shadow of doubt concerning the general fact of the postively heliotropic character of these larvae. It rapidly declines, however, during later life, tho still persists even in the hydroids themselves as numerous experiments by LOEB and others have shown.

Abnormalities. Of these I have found comparatively few, most conspicuous among which is that of the bifurcation of the planula at the (usually) oral end. This feature was quite common in the

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planulae of *Pennaria*, where it is described and figured, so that no special account of the phenomenon is necessary here, since it seems to be quite similar if not identical in the two. Sections of such planulae show that the bifurcation involves both ectoderm and entoderm and gastric cavity as well.

The fixation of the planula and development of the hydranth usually occurs within two or three days, tho it is sometimes, in specimens reared in small aquaria, considerably defered. As the period of fixation approaches the larval movement become less active, indeed rather sluggish; heliotropism largely disappears, or becomes negative in character. Finally the planula settled upon its broader, or oboral pole, fixes itself by a slimy secretion upon the bottom of the aquarium, or some fragment of sea-weed', or other support and finally ensheaths itself wholly within a delicate perisarc, which, like that of *Pennaria*, and probably most hydroid planulae, is so very delicate and transparent as often to escape notice. Then ensues a period of several hours, probably twelve to twenty-four, of quiescence, during which final metamorphosis into the young hydroid occurs. Excellent figures of these phases are given in ALLMAN's Gymnoblastic Hydroids, tab. 13, figs. 11—16. With the budding of the tentacles the young hydranth ruptures or dissolves, the terminal perisarc obtrudes itself, expands its tentacles, having in the meantime acquired a mouth, and begins the serious struggle for existence.

Naples, June 23, 1903.

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Explanation of the Figures.

Plate 14.

Fig. 1. Long-section of stem of *Eudendrium racemosum*. *O* eggs in various stages of growth. *Gc* glandular cells.

Fig. 2. Long-section of stem of *Eudendrium dispar* Ag. *O* eggs.

Fig. 3. Cross-section of stem of *Eudendrium tenue* Ag. *O* eggs.

Fig. 4. Long-section of stem of *Eudendrium ramosum*. *Gc* glandular cells, *ec* ectoderm, *en* entoderm, *o* eggs.

Fig. 5. Section of gonophore of *E. ramosum* with contained egg, showing the yolk spheres *ys*, nucleus, *N*, with wrinkled outline.

Fig. 6. Section of gonophore showing nucleus in process of migration and apparently devoid of nuclear membrane.

Fig. 7. Margin of egg in section, showing aggregation of cytoplasm about the disintegrating nucleus.

Fig. 8—13. Various stages in superficial cleavage of eggs. Figs. 8—11 sketches of a single eggs within an interval of half an hour. *G.p* outline of gonophore.

Plate 15.

Fig. 14. Cross-section of egg within gonophore, showing three nuclei near periphery.

Fig. 15. Section of egg showing yolk balls, and irregularly disposed nuclei *N*, *N'* position of nuclei in next section of the series. About 300 : 1.

Fig. 16. Section of egg which has passed all phases of surface cleavage, showing superficial aspects of nuclei, together with others within the yolk masses. 250 : 1.

Fig. 17. Section of egg in stage similar to preceding.

Fig. 18. Section of egg somewhat further advanced, showing primitive ectoderm *ec*, and nests of nuclei *Nc*, together vacuolated condition of superficial cytoplasm.

Fig. 19. Section of egg at about same stage as preceding, showing something of the cytoplasmic reticulum *Rt*, at several points.

Fig. 20. Somewhat different aspects of similar stage in another egg.

Fig. 21. Partial section, showing in more detail the cytoplasmic reticulum, and chains of nuclei. 520 : 1.

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Fig. 22. A. portion of section, showing a nest of nuclei with in a yolk-ball. B. a single nucleus fully organized, from same section. About 1000 : 1.

Fig. 23. Two nuclei *a* and *b*, in amitotic division.

Fig. 24. Cell in mitotic division.

Fig. 25. Several cells from same section in mitotic division.

Plate 16.

Fig. 26. Section of an eggs showing fully developed ectoderm at *Ect* and only partially developed at opposite pole. Glandular cells at *G. c.* No signs as yet of entoderm-formation. 300 : 1.

Fig. 27. Portion of ectoderm more highly magnified and showing cytologic character of the gland-cells *G. c*, lower with apparently two nuclei.

Fig. 28. Portion of section showing aspects of the embryonic development shortly before the escape of the planula. The vacuolated condition of the ectoderm at this time is very conspicuous.

Fig. 29. Two of ectodermal gland cells highly magnified.

Fig. 30. Aspects of ectoderm at about time of escape of the planula.

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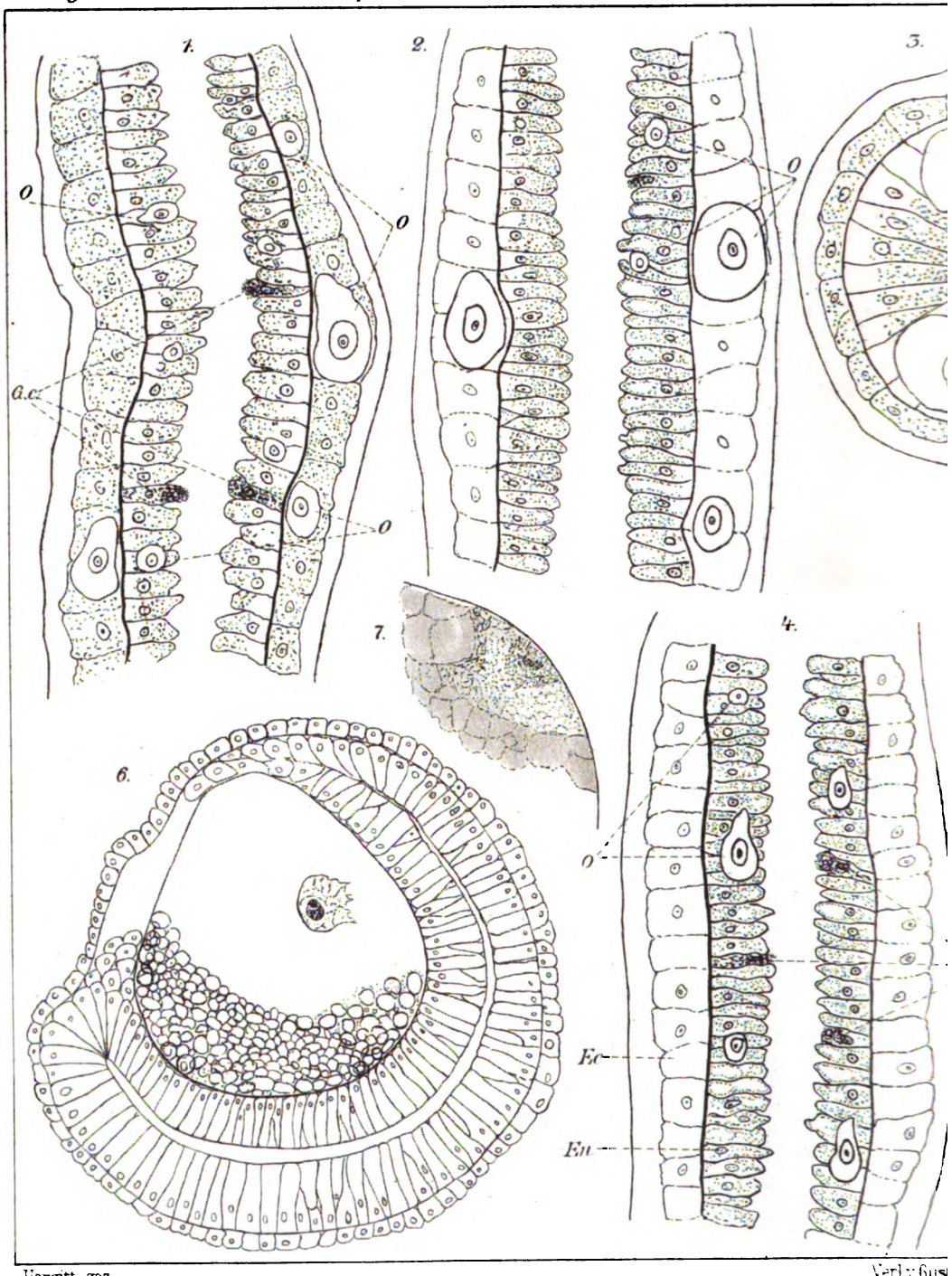

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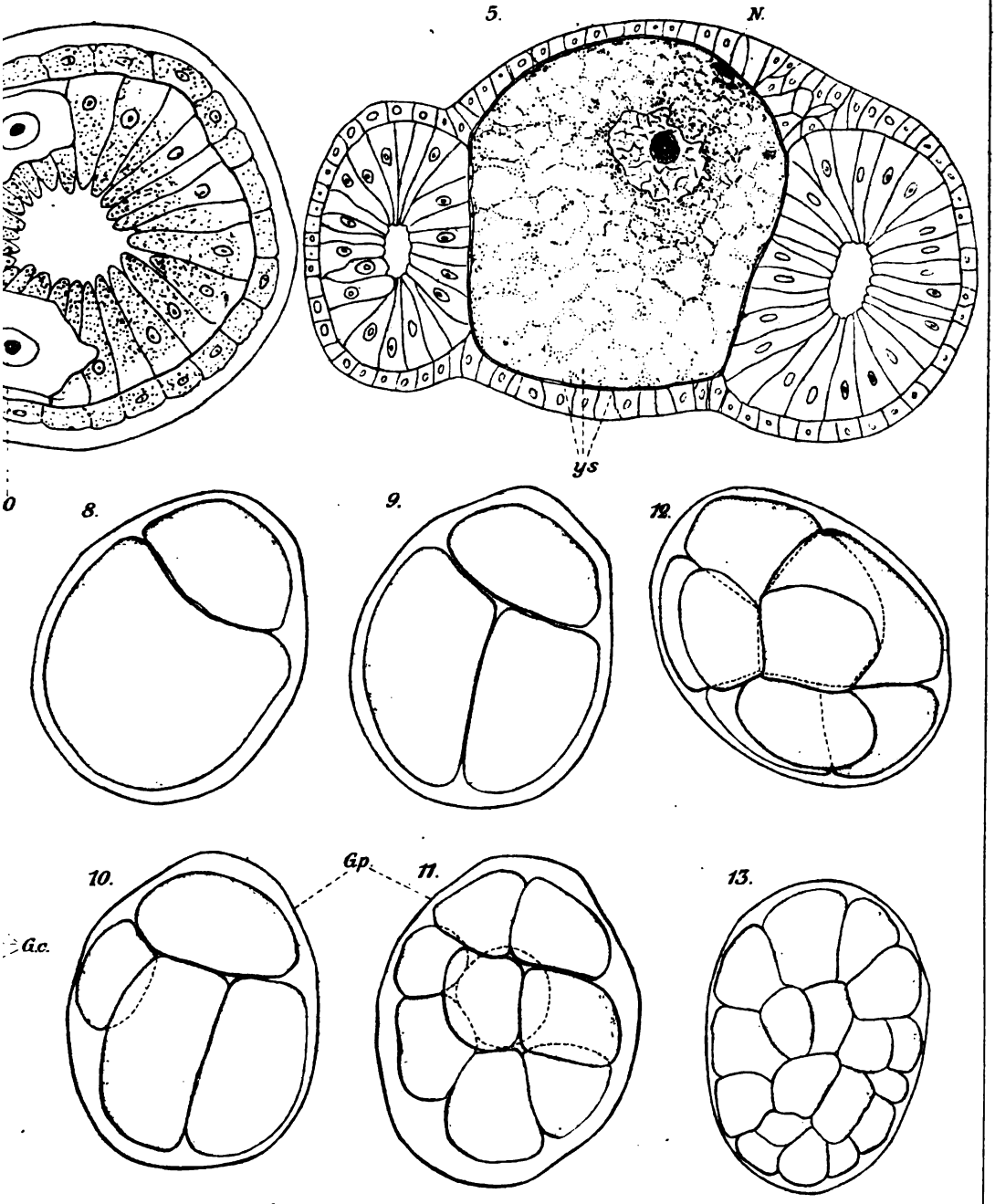

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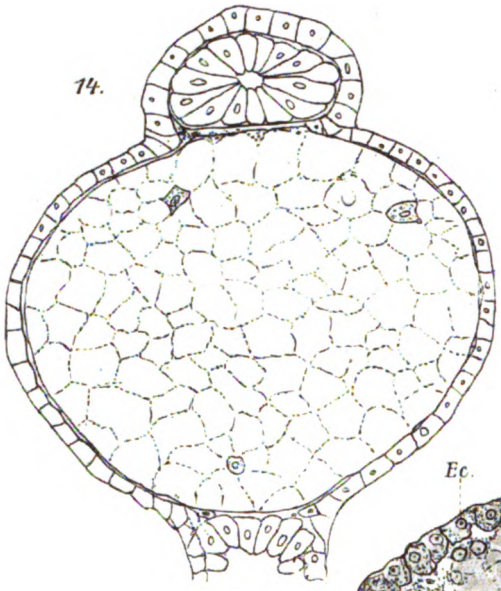

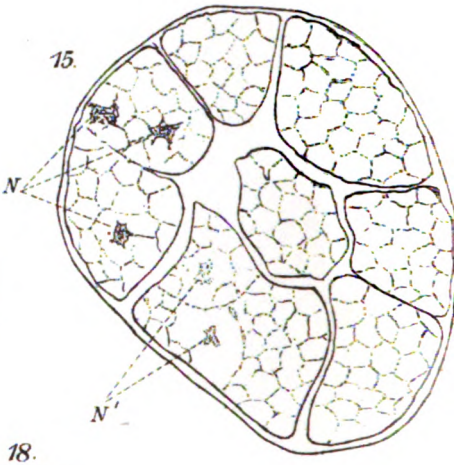

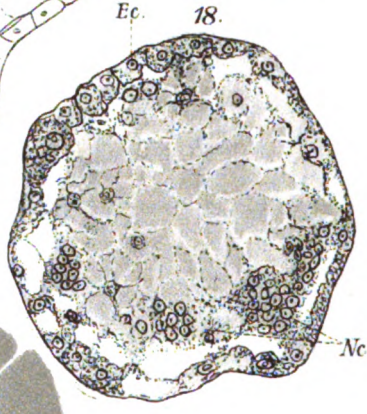

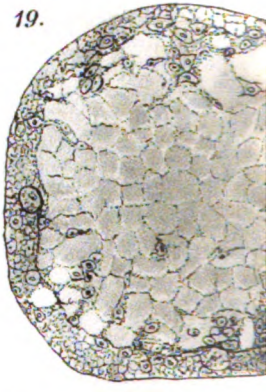

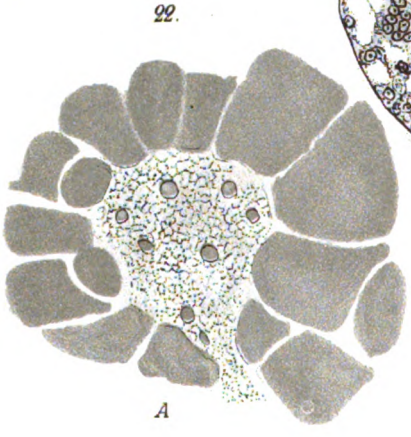

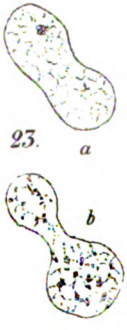

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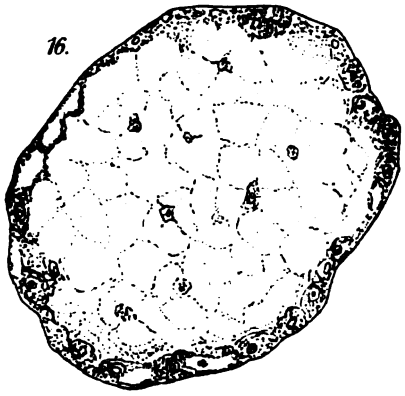

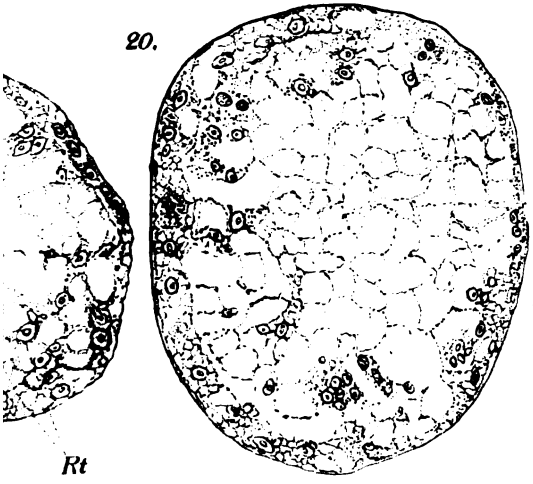

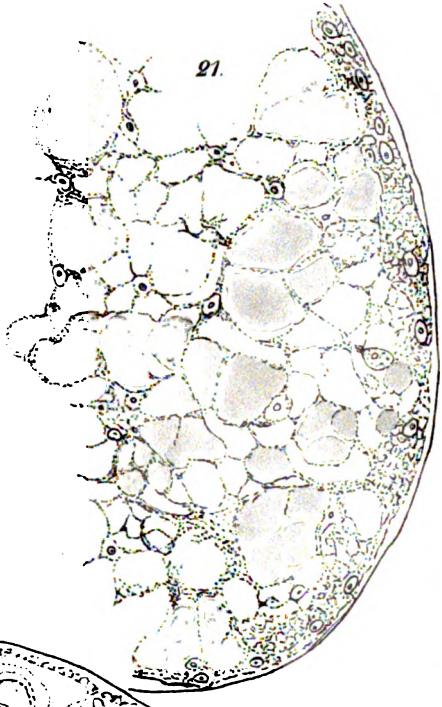

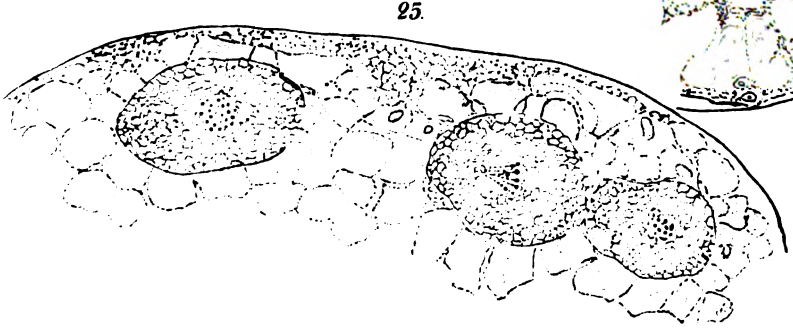

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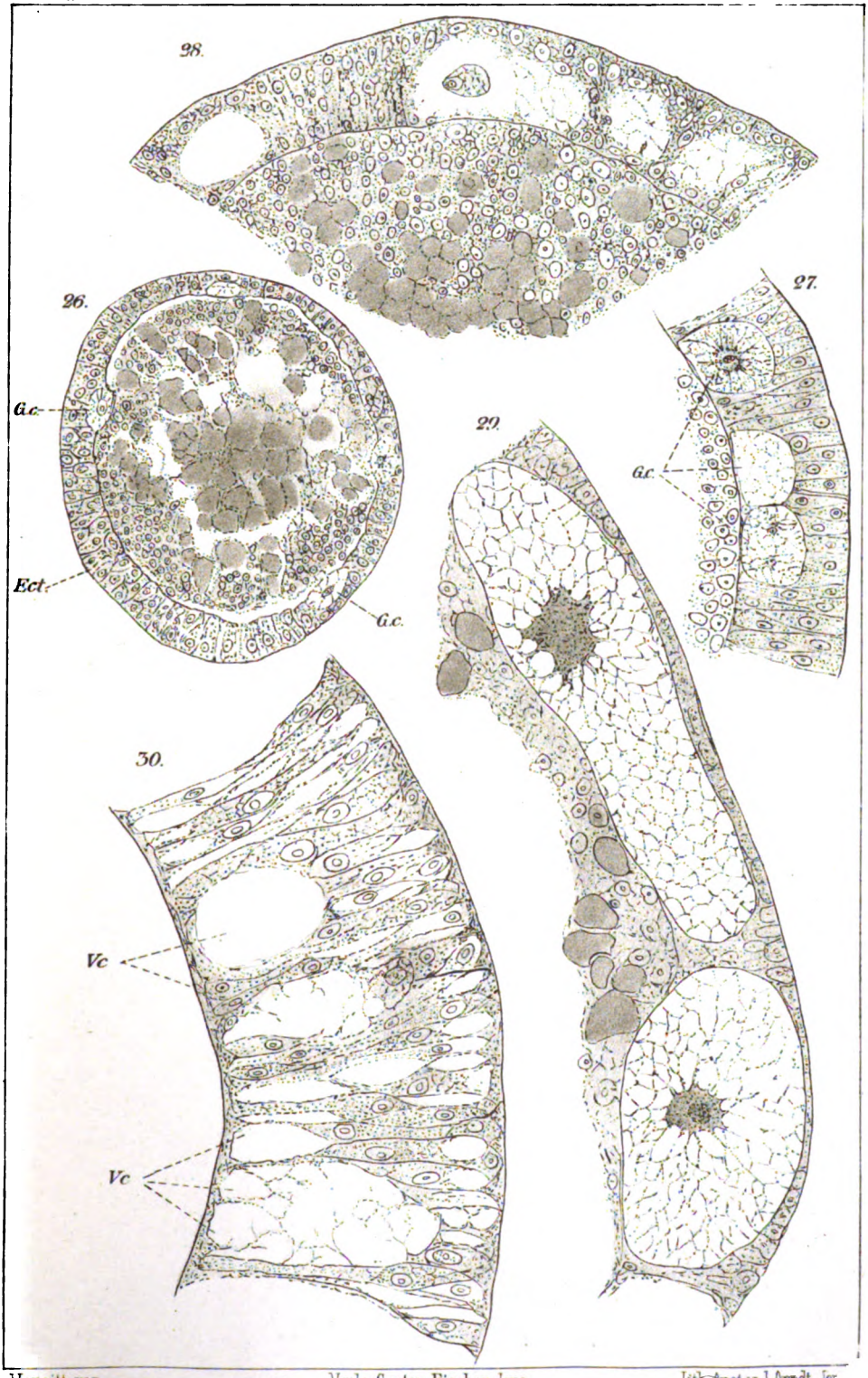

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The Early Development of Pennaria tiarella McCr.

By

Chas. W. Hargitt[1]).

With Plates XXIV—XXVIII.

Eingegangen am 15. April 1904.

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I. Introductory.

In a previous contribution on the natural history of *Pennaria tiarella* by the present writer '00, occasion was taken to present some observations upon the early development, but chiefly of a tentative and preliminary character. It was fully expected that a full account of the entire development would be made at a very early date, but the imperfect preservation of material collected at the time

[1]) Contributions from the Zoological Laboratory, Syracuse University.

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rendered necessary a postponement till such time as additional collections might be made and more satifactory methods of preservation applied, neither of which occurred till the summer of 1902, since which time extended absence from my laboratory has further delayed its prosecution.

It was also hoped that during the sojourn of several months at the Zoological Station at Naples I might be able to secure material for the study of *P. Cavolini* with a view to critically compare it with the former. In this however I was doomed to disappointment, since the apparent lateness of the breeding season of the latter during the past summer and the extreme scarcity of material made it impossible to make any observations upon its development. While regretting that owing to these conditions, as well as to farther disappointment in the results of preservation of material obtained at Woods Holl, certain aspects of the problem may be less conclusively determined than was expected, it seems better upon the whole to submit such results as have already been obtained, leaving for subsequent inquiry such phases as may call for additional attention.

II. Methods.

It has already been intimated that methods of fixation and preservation formerly employed proved unsatisfactory in many respects. It is therefore important that certain estimates given on modes of fixation in the previous paper (op. cit.), be somewhat modified. I formerly expressed some slight preference in favor of KLEINENBERG's picro-sulphuric acid, or picro-acetic acid. While for some phases these reagents afford fairly good fixation, for the cytologic study of the internal conditions of the egg in very early maturation and fertilization they have been almost worthless. The repeated experiments on fixation of these eggs have shown them to be unusually difficult to handle by any of the more common methods, tho in many cases fairly good results were obtained provided the material was used very soon after preservation. If left for six months or more it proved very unsatisfactory.

In my later experiments I have found the various corrosive sublimate solutions to give upon the whole the best results, particularly if the alcoholic solutions were used. It was not found that the addition of acetic acid to these preparations had any appreciable value, tho in a few cases there seemed to be some slight advantage

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in uniformity of results where glacial acetic acid was added to the alcoholic sublimate to the amount of 5%.

FLEMMING's strong solution usually gave excellent fixation, but, as noted in the previous paper, rendered subsequent staining difficult and more or less unsatisfactory. HERMANN's solution was similarly good, tho with similar drawbacks as to subsequent operations of staining, etc.

For immediate use I have obtained excellent results in fixation with a 10% solution of formol in sea water; in some cases indeed a 20% solution in the same medium proved very satisfactory. In a few cases I have tried the formol in full strength, but it seemed to operate with much less vigor and with apparently indifferent subsequent utility. In a few cases I have obtained excellent results from a mixture of equal parts of a saturated solution of corrosive sublimate and 10% solution of formol.

The eggs of *Pennaria* seem not to show that peculiar reaction to any one or few fixing agents in a specially favorable way as is the case with many other eggs, such as those of annelids, molluscs, etc. The same is likewise true of the eggs of *Eudendrium*, *Clava*, and others having large stores of yolk. I have also found that in such eggs there is an apparent deterioration in cytologic characters from extended preservation. If used within a few weeks or even a few months the reagents above indicated have proven fairly satisfactory, while if kept in storage for a longer time they have proven increasingly refractory. This has been found true no matter whether preserved in alcohol or formalin, whether kept in vials with glass stoppers, or with cotton plugs, or sealed with paraffine.

What has just been said as to fixation and preservation applies in general to staining reactions. Material freshly killed may be fairly well stained by any of the commoner nuclear stains, even when stained in toto. To obtain the best results in details of mitotic figures, chromosomes, etc., it was found necessary to stain with HEIDENHAIN's iron haematoxylin on the slide.

Many attempts to obtain satisfactory differential staining of the entire eggs for surface study by methods which have proven so exceptionally good with the eggs of molluscs, annelids, etc., have been most disappointing failures. CONKLIN's picro-sulfuric-haematoxylin gave the only results worthy of mention, and even this was of small value.

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III. Origin and Growth of the Eggs.

In a paper on the morphology of *Pennaria* SMALLWOOD ('99), gave a brief account of the growth of the eggs in connection with some account of their origin. In the various details of my own work it has become necessary to review in some measure this feature and I have been able to confirm SMALLWOOD's observations in all essential points and in a few details to extend them as well as to compare them with the corresponding phases in the history of the egg of *P. cavolini*.

In most respects these species show almost identically the same aspects of origin and growth. While in their general features the medusae of the two species are very similar in shape, size, etc., I have not been able to determine that the medusa of *P. cavolini* ever becomes free from the hydroid or leads in any sense an independent life. In the original account given by CAVOLINI (1785), he describes the larvae as escaping from the attached medusa. So far as I am aware the medusa has never been described, and it has probably rarely been seen in a free state, yet I am constrained to believe that in some cases it may share with *P. tiarella* a brief free-swimming phase during which it probably discharges the sexual products in quite a similar way. I find it going forward in development and becoming so loosely attached that its connection could hardly fail to become broken at times during the expulsion of the sexual products, and this motion could hardly fail to give to it at least a brief period of free life. Preserved specimens in a perfect condition of development moreover never have shown any evidence of any development of the eggs or of larvae. It would seem therefore quite probable that were careful search made for it during the particular period of its maturity it would be found, and would I believe conform in this respect with the former species.

The eggs originate in the ectoderm of the manubrium just as as in *P. tiarella*, and grow in much the same way, tho it should be mentioned in this connection that from the first the structure of the egg is of a distinctly reticular character, and continues thus up to the period of full growth, while the egg of *P. tiarella* has, as I have elsewhere shown, a definitely granular structure, during growth and early development.

The number of eggs to reach maturity is about the same in both species, averaging from six to eight, rarely more, tho' often

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less. If we consider the immense number of primitive ovarian cells it might seem that a much larger number would reach maturity, as is the case with many other similar medusae. When however it is borne in mind that the eggs of *Pennaria* are relatively very large it will be seen that the number must be correspondingly smaller.

The same phenomenon of cannibalism among these eggs occurs in both species, the growing eggs feeding upon their less fortunate fellows, so that by the time the medusae are ready to discharge them almost all of the primitive ova, or pseudocells, as they have been designated by BRAUER, have been devoured, or absorbed.

There is an apparent difference between the two species in that in the one, *P. tiarella*, there seems to be developed among the growing eggs definite pseudopod-like processes which insinuate themselves among the pseudocells and engulf them entire, while in the other these lobe-like pseudopods are replaced by delicate thread-like filaments which insinuate themselves among the eggs and seem to act like disintegrating ferments, the pseudocells appearing to dissolve under their touch and to be absorbed in a more or less liquefied condition. I have not found any evidence of the bodily engulfment of these primitive ova such as SMALLWOOD has found in the former species, and as DOFLEIN ('96), ALLEN ('00), and MAY ('03) found among the Tubularidae.

It seemed rather characteristic in the observations just cited to find, particularly among the species of *Tubularia*, a very prevalent condition of amitosis among these pseudocells, even after their absorption by the growing eggs. I have found nothing whatever of this character in *Pennaria*. The pseudocells appear to simply disintegrate or break down as under the influence of a ferment, as already suggested, showing no distinguishable cytogenic indications of any sort. May it not be that in this phenomenon as found in *P. cavolini* we have a clue to the more highly reticular character of the egg cytoplasm, already referred to, the reticulum thus serving as a meshwork for the retention as well as absorption of the liquified ova?

In this connection may be pointed out a phenomenon not unusual among the eggs of Hydromedusae, namely, a considerable variation in size, some becoming almost if not quite, twice as large as others in the same medusa. This may be probably accounted for by the peculiar mode of nutrition of the growing eggs as already described. Those to get the start maintaining it during growth, and since in all cases where the ephemeral history of the medusa exacts a simul-

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taneous discharge of all the eggs, those having been late in the original growth impulse must mature more rapidly and therefore be of smaller size at the time of liberation. This difference in size may possibly be due in certain cases, as pointed out by ALLEN (op. cit.), to the fact that occasionally two growing eggs which come into close contact during growth may actually fuse bodily, and that when this occurs during later growth must result in a single gigantic egg, which undoubtedly must give rise to a correspondingly large larva, such large larvae not being specially rare.

It would be a fruitful subject for inquiry as to whether in these cases of fusion the two nuclei likewise fused into a common large nucleus, and if so what changes occurred during the process of maturation. Or if there was no fusion what changes occur during the process of the disintegration of the one or the other, or whether indeed both might not share the same fate, involving a sort of fragmentation, a subject further considered in another connection.

During growth the eggs naturally have a more or less flattened or saucer-like shape from their position within the ectoderm of the manubrium, and with irregular marginal lobings due to the variously shaped absorbent processes, similar in some respects to the growing egg of *Hydra*. But as the growth approaches completion the eggs withdraw the processes, become definitely spherical, acquire the distinctive pigment and form conspicuous bodies within the bell of the medusa, as shown in a former paper (op. cit.).

IV. Maturation and Fertilization.

In view of the opacity of the eggs of these species any satisfactory observation upon them during life is extremely difficult, except that having reference chiefly to superficial aspects. Concerning the latter mention may be made first of the polar bodies. Whether from their formation before the discharge of the eggs, or from their ephemeral character, or from reasons unknown, I have been able in only a few cases to definitely distinguish these bodies. In one case shown in Figs. 35—40 what seemed both from general aspects and position and the relation of the subsequent cleavage, to be a polar body was distinguished and followed during the entire period of the early cleavage. It was not possible however to distinguish within these corpuscles any indications of nuclear matter, tho as will be shown in another connection this may not be unusual. From sub-

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sequent studies of the cytologic conditions found I am constrained to believe that probably these bodies are usually formed just prior to the discharge of the egg, and that moreover the membraneless condition of the eggs renders them quite ephemeral.

Among hundreds of eggs which have been studied in sections it is, however, almost as rare to find clear evidences of the internal phenomena of maturation. In Fig. 47 is shown one of a very few such cases which have come under observation. And I may say that the same is likewise true of the eggs of several other hydroids and medusae which have come under my notice. In the section to which reference has just been made the egg had been killed in FLEMMING'S solution and stained on the slide with HEIDENHAIN'S iron-haematoxylin, but not the slightest indication of chromatin could be found within the nuclear area. This condition seems to prevail for some little time both prior to, and following, the direct process of maturation, and indeed it seems to continue for some time following the ingress of the sperm. Sections fixed within fifteen minutes after artificial fertilization rarely showed distinguishable traces of either egg or sperm nucleus, or chromatic elements.

In this connection may be pointed out what seems somewhat noteworthy, namely the apparent dissolution of the nuclear membrane and the subsequent disintegration of the nucleus shortly before the discharge of the egg.

Sections through the entire body of the medusa at this time showed in almost every case the migration of the nucleus to the outer periphery of the egg, and in many cases its apparent protrusion against the retaining ectoderm of the manubrium, where by degrees it became more and more indefinite in form and structure and finally lost entirely its chromatophilous qualities. Just what may be the significance of these nuclear phenomena does not seem to be at all clear. That they may sustain some relation to the nuclear metamorphoses associated with maturation does not seem improbable, notwithstanding the apparently anomalous features involved.

Similar observations have been made by others at various times and upon various eggs.

KOCH ('87), has called attention to a very similar condition in the egg of *Gorgonia*, in which following fertilization and including the earliest phases of cleavage, it is impossible to distinguish the nuclei. KOCH is disposed to regard the phenomenon as in some way due to the direct effects of fertilization.

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As I have intimated above, and have likewise shown in connection with the development of *Eudendrium* ('04), it must be due, at least in these forms, to conditions preceding fertilization.

HICKSON ('94), has described almost identical phenomena in the development of several alcyonaria, notably *Distichopora*, and ascribes it without hesitation to a process of nuclear fragmentation, citing in support of his view considerable evidence from widely different sources and of high character.

KINGSLEY ('92), has noted a somewhat similar condition in the egg of *Limulus*, but ascribes it to some chemical change which occurs at this time in the constitution of the egg protoplasm.

MAYER ('77), has also observed the same conditions in the early history of the egg of *Eupagurus*. He finds that the germinal vesicle disappears while the egg is still in the ovary, thus leaving the newly laid egg enuclear. Just prior to its disappearance he finds it passing from its normal place at the center to the periphery of the egg, where it may be distinguished within a protoplasmic area. This migration to the periphery he regards as abnormal, suggesting that it probably was indicative of the disintegration of the egg.

As will be seen MAYER'S view has much in common with that of KOCH (op. cit.). Like this observer MAYER considers this disappearance as following upon fertilization, and probably in some manner caused by it. As I have already shown this is certainly not true in the case of *Pennaria*. Indeed, as is well known now, the supposed abnormality of the migration of nucleus to the periphery of the egg is a very familiar phenomenon and involves nothing unusual or abnormal. Its total disappearance, which MAYER claims to have carefully demonstrated, is only one more among many similar cases which have been observed.

While not undertaking to cite numerous other cases of similar import, some of which I have elsewhere reviewed, I cannot pass without considering in some detail a series of extremely interesting and important experiments and observations recently made by LILLIE ('02), several of which have an intimate bearing upon the subject here under discussion. This is particularly the case with those portions dealing with nuclear phenomena involved in the breaking up of this organ and the dispersal of its elements throughout the cytoplasm. I can best present LILLIE'S views by quoting directly his own words. Discussing this aspect of the matter he says, »The chromosomes are in process of breaking up, the granules are scatter-

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ing throughout the cytoplasm, the larger portions, however, remaining in the center. It is not possible to believe that the scattered particles are ever entirely gathered in again. A great part of the granules must be lost in the cytoplasm, and never form part of the nuclear area that is seen later in each egg. In this case there can be little doubt that diffusion of nuclear matter throughout the cytoplasm takes place. The ultimate effect of this is, of course, problematical; but it may be significant that this experiment was one of the most successful .... of the entire series. Even the smallest discernible particles of chromatin exercises, apparently, a liquefying effect on the cytoplasm, and thus appear to lie in vacuoles, a condition analogous to the return of the nucleus to its resting condition.«

In citing these observations in this connection I do not overlook the fact that they pertain primarily to conditions which are more or less artificial, and which may, to a greater or less extent, be supposed to have induced them. In other words, that the results, while similar in many respects to phenomena occurring naturally in certain cases, are analogous rather than homologous. On the other hand, it should not be overlooked that so far as end results are concerned in the development of the embryo they have not in any appreciable way interfered with normal processes. And if such be a fair inference it seems to me we may safely presume that conditions in no appreciable degree different, which have been cited by other observers under admittedly normal influences, such as those previously noticed, are to be accepted as facts whatever interpretation we may choose to give to them.

So far as the case of *Pennaria* is concerned the facts are not altogether convincing. Whether there may not be in certain cases substantial evidences of fragmentation and subsequent reorganization of nuclei from such scattered fragments, while in others it may be possible to trace a direct lineal connection of every nucleus throughout the embryonic history, without involving any insuperable contradiction or difficulty would seem a fairly safe presumption. In any case there can be no possible doubt as to the fact, that during a certain clearly defined period in the earlier history of this egg some change, chemical or physical, or both, supervenes which renders impracticable any differential demonstration of those nuclear phenomena which during this period are usually easily and clearly distinguishable. It is not impossible that this may be chiefly chemical, due to some intricate

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processes of metabolism, during which neither basic nor acid stains have any effects upon just those elements with which we are concerned. As is well known, such conditions do occur in varying degrees, tho not to such extent as to involve any considerable doubt or discredit upon the value of modern staining methods in general. On the other hand, if we may assume the operation of disintegrating factors during the complex metabolism and molecular readjustments involved within this period of intense protoplasmic activity such as result in the breaking down and dispersal of the chromatic material, as the observations of LILLIE above cited clearly seem to demonstrate, it is quite easy to explain the conditions here under consideration.

Of some degree of significance in this connection is the fact that following this non-staining, or achromatophilous period there is often found what, to all appearances, seem to be minute nuclear vesicles, in some cases singly, oftener in groups, or nest-like clusters, as shown in Figs. 46, 48, 49. I was at first disposed to consider these vesicular nuclei as indicative of amitotic division, and there are not lacking other evidences which might be considered as confirming this view; but upon the whole the conditions first mentioned, namely, that of nuclear or chromatin vesicles, better harmonize with the assumption of a previous nuclear fragmentation, followed later by a corresponding period of nuclear reorganization.

METSCHNIKOFF ('87, p. 29) has called attention to somewhat similar nuclear conditions, and has in several of his figures shown very similar nuclear features, but offers no special explanations as to their origin or significance. Indeed concerning certain aspects referred to he expressly says »Die Bedeutung dieser Structurverhältnisse ist mir unbekannt geblieben«.

In Fig. 48 will be noted some three well defined nuclei, all in the resting stage, one perfectly typical, another equally so except for the minute chromatin vesicle just to one side of it, while the third is composed of several nuclear structures, comprising a nest of vesiculate bodies of varying size. All these nuclear centers occurred within two consecutive sections, and were the only nuclear structures in the egg. The fact of there being three, an odd number, is also significant. No traces of asters or other nuclear features were distinguishable.

In Fig. 49 is shown a somewhat different condition, possibly associated with the formation of the polar body, which is shown at

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the surface. In the nucleus involved in the phase of maturation here shown we have the same vesiculate condition, apparently passing into the typical resting stage following maturation, with the aster still showing at one pole of the nuclear area.

The presence of another conspicuous nuclear center in close proximity to the former, both occurring in the same section, and as in the previous case being the only nuclei to be distinguished in the entire egg is significant. There could not be discovered any evidence that this spindle was in anywise related to a sperm nucleus, no sign of that body being apparent. To all appearance it seemed to be an ordinary nucleus in the process of mitotic division. It presents the interesting coincidence that when the division is complete and the nucleus has passed into the usual resting stage that we shall have here, as in the former case, an odd number of nuclei. Whether the coincidence is anything more than incidental is perhaps doubtful, yet none the less interesting.

In Fig. 50 is shown a series of nuclear characters still somewhat different from either of the former. The most remarkable aspect of the present series is the elongated, clavicular-like nucleus, which, as will be seen, is in all essentials, save that of shape, a typical resting nucleus. While such a nucleus among certain Protozoa would not be an occasion for remark, it is more or less anomalous among Metazoa. Shall we regard it, in its present aspects, as definite and final in form, or may it not be a stage in nuclear metamorphosis, passing from the condition of a chain-like series of nuclear-vesicles into a connected whole, gradually to assume the spherical condition? On the other hand, might it be interpreted as a phase in amitotic division, the somewhat unusual elongation being merely incidental? Upon the whole it seems to me that the former alternative is the more probable, namely, that in this as well as in the former series of vesicular nuclei, including such as those shown in Fig. 46 we have a somewhat remarkable series of nuclear metamorphoses, perhaps arising somewhat spontaneously from promiscuously distributed nuclear fragments in some cases, probably in others by direct derivation from a single original nucleus by ordinary processes of division.

Concerning the immediate phenomena of fertilization but little can be said. The opacity which as previously suggested rendered difficult any critical observations as to the internal phenomena of maturation, has operated with even greater force to prevent any critical study of fertilization of the eggs during life, and unfortun-

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ately the refractory character of the eggs in relation to fixation and staining has made almost equally difficult a satisfactory study of the phenomena by means of sections or other micro-chemical methods.

It has been generally assumed that in many cases among hydroids having sessile gonophores, such as *Eudendrium*, *Clava*, *Hydractinia*, etc., fertilization is accomplished by the sperms penetrating the ectoderm of the gonosac and thus getting access to the egg. As I have elsewhere pointed out this has seemed somewhat doubtful, tho I have not been able to clearly explain it otherwise. In the case of *Pennaria*, where the eggs lie just within the delicate ectoderm of the manubrium, a much more delicate membrane than that of *Eudendrium*, or indeed most of those just mentioned, I have not among hundreds of cases kept under close observation found a single case where there has been the slightest evidence of fertilization of the unlaid egg. I have taken special pains to isolate many specimens of female medusae just before the discharge of the eggs, as well as others during the process of their discharge and have never found any indications of fertilization of the eggs. That the fault was not in the eggs themselves is abundantly evidenced in that they were promptly fertilized after the introduction of sperms from the vessel where only a few minutes before the medusae had been swimming. It might be urged that in such cases as *Pennaria* whose eggs are freely discharged and therefore open to the easy ingress of the sperm, the latter are not adapted to penetrate even a delicate membrane such as is found here. While not without a measure of plausibility it seems to me to lack evidence. I have not been able to distinguish any apparent difference in the structural characters of these sperms as compared with those of other Hydromedusae.

As a rule the male medusae are liberated first and promptly discharge the sperms while actively swimming. The females usually escape shortly later, tho they often discharge the eggs while still attached to the hydroid. Fertilization takes place very soon after the eggs are discharged, and in specimens in the aquaria, or in the smaller vessels used for observation under the microscope, the sperms often surrond the eggs in such numbers as to give it a ciliated appearance and even give to it a rotary movement owing to their active movements. But aside from the slight surface contortion of the egg which may be sometimes seen during the contact of the sperms when under the microscope it has not been possible to trace the process. The eggs are membraneless when laid, and none is subsequently

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formed, tho a thickening or condensation of the ectoplasm which might be mistaken for such is often found during later cleavage.

As previously mentioned the character of the egg likewise renders impracticable any study of internal evidences of fertilization in the live egg.

Furthermore, the condition of the eggs resulting from fixation has proven such as apparently to render almost equally difficult any satisfactory subsequent study of the internal phenomena of fertilization. It has not been difficult to demonstrate the presence of the sperm head in eggs fixed within from fifteen to thirty minutes after the probable time of entrance, but the course of the sperm within the egg I have not been able to trace in a single instance. It apparently leaves no track whatsoever in the cytoplasm. Nor have I been able to demonstrate the conjugation of the germ nuclei. The sperm nucleus, like that of the egg during maturation, seems to lose more or less completely its chromatophilous properties soon after its entrance, nor does it appear to recover these characteristics in any distinguishable degree, so far as I have been able to determine.

In Fig. 49 is shown a section comprising conditions found in two consecutive sections of an egg which appear to suggest fertilization features, tho of a somewhat anomalous, perhaps pathologic character. A study of adjacent sections show unmistakeably the final phases of maturation, one of the polar globules being shown at the surface, and the other appearing in connection with an adjacent section of the series. In the immediate region of the polar globule is shown a nuclear condition simulating, if not indeed being in fact, the return of the nucleus to a resting stage following maturation. Associated with it are to be seen three asters with two well-defined spindles, a condition well known in connection with experimental methods, as well as in cases of polyspermy. Whether the latter be the condition here may not be improbable, tho if so it would seem highly probable that among hundreds of eggs examined in section other similar cases should have been found, but this is the only instance noticed.

An interesting condition is also to be seen in the presence of another well developed amphiaster in the same section and at considerable distance from the former. To all appearances it was an ordinary nucleus in the metaphase of division, tho how to correlate it with the conditions of the former is not at all clear. Whether both may be in some way expressions of abnormal, or pathologic processes or conditions would seem not improbable, except for the

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fact of its apparent singularity among the very large number examined. Of course, it is not impossible that other similar cases might have been overlooked. Further than this statement of facts and suggestion of possibilities I have nothing to propose.

Conditions somewhat similar in some respects are shown in Fig. 48, and in less degree in Fig. 50. In the first will be noted the presence of three definite nuclei, all in the so-called resting stage, and all occurring within two consecutive sections, and moreover, the only nuclei found in the egg. A previous reference in some detail to these figures obviates further discussion of them here.

While these primary and more immediate phenomena of fertilization must therefore remain undecided and uncertain so far as this egg is concerned, it has been comparatively easy to demonstrate the more or less profound effects of fertilization upon the egg cytoplasm and its organization. In eggs which were fixed by the usual methods, some of which have been referred to in an earlier section, shortly following the entrance of the sperm, the cytoplasm exhibited various aspects of condensation, vacuolation, etc. indicative of the operation of conditions of tension or stress or other forms of activity. There were not lacking indications of cytoplasmic movements, or readjustments similar in some respects to those discussed in another connection under the head of »ectosarcal phenomena«, involving the formation of papillae, connecting threads, etc.

It had seemed at first that these conditions might have been induced by certain coagulating effects of reagents used in fixation, but its general occurrence only, or chiefly, during this early phase of development, and its insensible graduation into undoubted centers of definite cytoplasmic activity associated with early cleavage can leave little doubt that it is in reality a direct result of fertilization. In Figs. 43, 47, as well as in many others, may be seen representations of some of these internal aspects, though no drawing can adequately express the intricate and finely graduated conditions which occur more or less abundantly at this time.

Conditions very similar to these are shown in several figures by KOCH and METSCHNIKOFF, as discussed in another connection, which seem to justify the inference that probably the phenomena are more or less common throughout the entire group of coelenterates, particularly in eggs like those of *Pennaria*, in which there is a considerable amount of deutoplasm, giving to them a granular texture within a viscid matrix.

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V. Experimental.

In connection with the foregoing observations concerning nuclear fragmentation and associated phenomena should be considered certain experimental results obtained during the progress of the work. Some of these have been briefly noted in the previous paper already cited. These will only be referred to incidentally. The first has to do wholly with the relations of the medusae to light or darkness, and so far as the experiments go they seem to be but slightly or not at all affected by such changes, tho at the same time they have rather definite time relations in the matter of discharging the sexual products.

The second of the experiments had to do wholly with the influence of temperature in relation to the matter of discharge of the sexual products and to the rate of cleavage and associated phenomena. In both respects it was found that temperature had a very noticeable effect. The same was true as to the rate of development and degree of activity of the larvae.

It was of a third class of experiments that most interest was involved, namely, those concerning the capacity of fragments of eggs to continue development and to produce entire embryos. The presence of ectosarcal globules upon the surface of many eggs, and their frequent detachment during cleavage without in any appreciable degree affecting the normal progress of development, suggested first the experiment of artificially removing still larger portions, and finally of dividing the various blastomeres during early cleavage. As is well known, the work of Roux, Driesch, Wilson, Morgan, Loeb and others had clearly demonstrated, tho in varying degrees, the development of partial or entire embryos from portions of the egg. Loeb had shown '94 that under the stress of osmotic pressure double or multiple embryos of sea-urchins might be produced. The ectosarcal phenomena, to be discussed in detail in another connection, showed many features in common with the results of Loeb, but in the experimental features under consideration here there was little which might be considered as analogous.

Following the excision of small fragments from the eggs at various stages which, as has already been suggested, had no unfavorable effect on the progress of development, various experiments were made in artificially dividing eggs during the several early phases of cleavage. This was naturally more easily accomplished during the first cleavage and in Figs. 62—69 Plate XXVIII, are shown some of the results so

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far as they appear upon the surface. As will be seen, each of the resulting halves behaved in a manner indistinguishable from that of normal eggs. These half embryos were followed through the entire process of cleavage and through the later metamorphoses into planula and polyp, and in every respect, size alone excepted, the processes were perfectly normal, as may be seen by a comparison with the figures of Plate XXV, where is shown the development of perfectly normal eggs.

Naturally the problem of the development of enucleated eggs came up in connection with these experiments and several attempts were made to test it. On the assumption that the nucleus occupied a more or less eccentric position in the egg, or at least a definitely localized place the eggs were artificially divided soon after fertilization by cutting them into fragments with fine scalpels, or in some cases with needles. One could hardly fail to secure thereby numerous fragments which must be wholly free from any nuclear matter, tho the opacity of the eggs made it practically impossible to be certain of the absence of nuclear matter in any particular portion. The particles were in some cases isolated and in others they were allowed to remain among the other portions in the dishes where the experiments were performed. The subsequent development of many of these fragments in each case would seem to warrant the conclusion that enucleated fragments of the eggs of *Pennaria* are capable of definite development into normal embryos.

These experiments were also performed by Dr. S. J. Holmes, of the University of Michigan, and with similar results, as he subsequently informed me.

In the light of some suggestions made in a previous connection concerning an apparent fragmentation of the nucleus in these eggs, a condition which has been rather strongly indicated by similar conditions found in the eggs of *Eudendrium*, as well as by cases cited from other investigators, the question has arisen, whether in the present experiments the supposed enucleated fragments were really such, or whether on the assumption of the fragmentation of the nucleus, every portion of the egg, even its smallest fragment, did not really contain nuclear matter, and that in sufficient amount to secure the definite organization of the cytoplasm and to carry forward the development to a successful issue. Evidently no decisive conclusion can be drawn until farther experimens have been made, but in the light of the facts of nuclear behavior during the processes of matur-

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ation, we may well doubt whether, in the present case, at least, our supposed enucleated fragments were such in fact. And if such a contingency be possible in the present instance may it not be true in other similar cases? Of course, in cases where the character and organization of the egg are such as to enable the experimenter to clearly remove the nucleus and thus exclude its possible relation to the subsequent development the above inference would not hold. And it might be urged that with one such unquestioned case in evidence, the more rational conclusion would be, in such instances as that under consideration, to ascribe to it similar causes and to make similar inferences. While such argumentation may seem fairly logical and satisfactory it should not be overlooked that from the known behavior of nuclear matter under other conditions and the importance of its rôle in the phenomena of cell physiology, we must allow to the previous suggestions at least a reasonable plausibility, and at least a suspended judgment until such time as farther evidence may warrant a different attitude.

VI. Ectosarcal Phenomena.

A feature more or less conspicuous during the earlier development of these eggs, and which is more or less definitely ectosarcal in character calls for consideration in this connection. While not a constant feature in all eggs, it is nevertheless so fairly prevalent as to constitute a more or less remarkable characteristic.

In general it is characterized by the occurrence of certain papilliform processes, projections or lobings of the superficial portion of the egg cytoplasm, remotely comparable with somewhat similar features in the egg of *Hydra*. Chiefly for convenience of description their more distinctive aspects may be discussed under the following heads, tho it should be understood from the outstart that they graduate more or less imperceptibly into each other.

1. Papillae.

These are for the most part small, knob-like projections at one or several portions of the egg, usually just prior to the superficial signs of cleavage, or very early in its progress. As a rule they are purely ectosarcal, tho at times they seem to involve the deeper portions of the cytoplasm as well. In character they are generally minute, spherical bodies, more or less refractile, sometimes resembl-

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ing polar bodies, but wholly devoid of chromatin or other nuclear matter. As shown in Fig. 43, they may at times be confined within a delicate ectosarcal film which has something of a membranous character. These papillae may increase in number or size or in both; may be more or less transient, or may persist for several hours after which they are usually resorbed, or more rarely cast off entirely from the egg and thus lost. In their origin and formation they are often capricious and usually quite slow, so that it is like watching the movements of the hour-hand to distinguish their development, tho occasionally a relatively more rapid growth is noticeable, but I have not been able to distinguish even under the high powers of the microscope anything like currents of cytoplasm involved in their formation.

2. Threads.

These are very common and conform more nearly with such features in many other eggs. They usually appear as delicate filaments of ectosarc extending from one blastomere, or pseudopod-like process, to an adjoining one and would seem to serve as connecting, or binding structures uniting the several portions of the egg into an organized body. It may be doubtful, however, whether this alone fully explains them as they not unusually dissolve, or become discontinuous, and often are wholly resorbed, as are the former. In some cases several of these threads may form in close relation to each other and thus continue for some time, finally to disappear as before. Sometimes they become spun out into fibrillae of extreme fineness all but invisible under high powers, or on the other hand they may gradually assume a heavier and coarser aspect and broaden out into film-like structures.

3. Films, or Bridges.

Several of these structures are shown in the various figures, particularly such as 8, 9, 15, 16, 56, 57.

As will be seen, the second and third of these structures pass by insensible degrees into each other, and are probably but differing aspects of one and the same fundamental phenomenon. The latter usually appears during the very early cleavage phases and forms sheets of ectosarcal matter, more or less homogeneous in appearance, and usually very thin and delicate. It not unfrequently happens that from some apparently intrinsic, vital readjustments of the cytoplasm of these films small openings appear here and there,

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which may enlarge, finally breaking through one edge of the film, or in other cases fusing again into a continuous sheet.

Concerning the significance of these films little can be said more than was suggested in connection with the former. That they may in some measure serve as bonds of connection, or »bridges«, through which matter may pass from one blastomere to another, or by means of which there is made possible a more intimate organic connection of the several blastomeres, may be highly probable. Whether this is their chief, or only function may be somewhat doubtful, since their absence, or at least small development in many, perhaps the majority of cases, would militate against this view. It should be noted that during later cleavage stages they are much less conspicuous, indeed apparently absent in many cases.

Phenomena of probably similar significance, at least in some degree, have been described by several investigators, notably by G. F. ANDREWS ('97), E. A. ANDREWS ('98a, '98b, '98c), ZIEGLER ('98), WILSON ('00). G. F. ANDREWS was among the first to describe these phenomena, which she designated as »Spinning Activities of Protoplasm in Starfish and *Echinus* Eggs«; and related to extremely tenuous, threadlike, or filose strands of protoplasm, apparently associated with the maturation and early development of the eggs of these animals.

Similar observations upon the same eggs were made later by E. A. ANDREWS, who also described similar phenomena ('98a), in connection with the eggs of *Cerebratulus*. These were followed by observations ('98b), on other »metazoan eggs«, and still later upon the eggs of *Hydra* (98c).

ZIEGLER ('98), described very similar phenomena in the eggs of *Beroe*. In 1900 C. B. WILSON confirmed and extended the observations of E. A. ANDREWS upon the eggs of *Cerebratulus*. In this case the phenomena were confined for the most part to the processes of maturation, involving chiefly the polar bodies, or portions of the egg in close proximity, and probably induced by the activities of these bodies. This observer also records papilliform processes as occurring at other portions of the egg, but chiefly on abnormal, or unripe eggs. These latter have some aspects in common with those of *Pennaria*, except that in the latter case they are not peculiar or common in unripe eggs, indeed quite otherwise, as a rule. WILSON regards these activities as essentially expressions of nuclear activity involving both the egg nucleus and polar bodies.

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ANDREWS regards the phenomena as among the most characteristic and fundamental of protoplasmic activities, in some cases connecting the blastomeres and cells of larvae, and always serving to coordinate the activities of protoplasm and parts of the developing embryo, the latter being the significance ascribed to them when first described by G. F. ANDREWS.

Concerning the papillae it is difficult to offer any very satisfactory explanation. How they could serve in any way to coordinate the complex physiological changes which are involved in the egg during this period it is extremely difficult to imagine. If all were resorbed we might interpret their occurrence as external expressions of internal stress or tension associated with the cytoplasmic activities coincident with the process of fertilization. But it is not unusual to see these globules pinched off and entirely lost to the egg. However the fact of their occurrence in close relation to the processes of fertilization is strongly suggestive of some causal connection therewith. This is further indicated in the general fact of their replacement soon after cleavage is fairly begun by the films and threads previously considered, tho it should not be overlooked that they may occasionally persist for some time after cleavage has been under way. Figs. 43, 44, 56, 62, show outlines of these features in the egg before and during cleavage. In the latter case it will be seen that these papillae are confined chiefly to one pole of the egg, while in the other they occasionally form at various points on the surface, tho even in these cases their occurrence is more frequently restricted to a limited portion of the egg surface. In Fig. 43 is presented a sectional sketch showing the appearance of these papillae and their relation to the nuclear areas which will further support the inference of some causal relation between the two.

It has been mentioned in an earlier connection that the entrance of the sperm often seemed to incite a slight torsion of the egg surface, or a constriction at the point of entrance. While the opacity of the egg made impossible any direct observation of internal changes following the sperm entrance, a study of sections show that more or less profound changes are involved, such as condensations and vacuolations of the cytoplasm, and as suggested above it may not be improbable that these papillae are but external expressions of internal activities, as cytoplasmic currents, nuclear readjustments, or similar phenomena.

As is well known, cytoplasmic movements within the egg during

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cleavage and differentiation have long been recognized and various explanations proposed, among the more suggestive of which, among earlier observers, are those of HERTWIG ('78), FOL ('79), MARK ('81), BÜTSCHLI ('94), WHITMAN ('87), referring to such phenomena as astral radiations, nuclear spindles, etc.

Among more recent investigations along these lines those of CONKLIN ('99, '02), LILLIE ('99, '01), and WILSON ('03), are noteworthy. CONKLIN has emphasized the importance of orderly movements in the protoplasm as a factor in differentiation in the egg of *Crepidula*, and LILLIE has likewise shown its relation to similar developmental features among lamellibranchs, and also to some extent in observation upon certain cleavage phenomena in the egg of *Chaetopterus*. WILSON has also discussed the same factor in his recent paper on »Experiments on Cleavage and Localization in the *Nemertine*-egg«. The very full discussion in details of the many recent observations along the same general lines renders unnecessary any extended account of the subject here.

Repeated examinations of the living egg of *Pennaria* under high powers failed to reveal the presence of definite cytoplasmic currents or similar movements. Nevertheless, it was possible to observe certain readjustments of matter about the nucleus, as also to a less extent in the formation of the ectosarcal characters noted above. Moreover, a study of sections of many eggs during the early phases of development afforded undoubted evidence of just such readjustments as would be involved in slow currents in a viscid liquid. An inspection of several of the figures previously cited as to ectosarcal and nuclear phenomena will clearly show these conditions. Fig. 70, which is a camera sketch of an egg during later development will also strongly suggest such a view.

Not only in these aspects, but in the more or less constant flattening of the egg during cleavage, as is clearly shown in many of the figures, including the evident amoeboid tendencies, there is more or less conclusive evidence of a condition suggesting the operation of some force like extremely slow motion of a viscous body under the influence of gravity.

The conditions presented however, are but expressions of still more fundamental and profound intrinsic changes within the cytoplasm itself. Whether these be chemotactic, as CONKLIN has suggested, or whether of some source of energy, the nature of which we are as yet in absolute ignorance, it is impossible to say.

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VII. Cleavage.

1. Early phases.

Cleavage is total, and at completion there is formed an ovoid embryo composed of a solid mass of cells of fairly equal size, constituting a morula, a sectional view of which is shown at Fig. 72 Plate XXVIII. Between the extremes of the embryonic history from the early cleavage to the formation of the morula are to be found the most erratic and anomalous exhibitions of developmental phenomena which have ever come to my knowledge, if indeed its counterpart has hitherto been known. It is not strange that with the mental pictures of such steady-going exhibitions as are found in the development of annelids, molluscs, etc., one should regard such monstrosities as are very inadequately represented in the various figures illustrating this paper as abnormal to the degree of being pathologic! And thus it seemed to me when first observed; and as pointed out in the earlier paper, the first batch of eggs were discarded as having »gone bad«. Such was also the verdict of several critical observers to whose attention they were called somewhat later, notwithstanding the fact that in the meantime there had been obtained several series of normal embryos as evidence of the normal nature of the development.

Since the publication of the earlier account I have taken occasion to reexamine the entire history of these eggs from their discharge by the medusa through the subsequent phases of development to the perfect polyp stage and am prepared to reaffirm every point advanced at that time and to furthermore insist upon the perfectly normal character of the development, — normal for this animal. As the warrant for this somewhat dogmatic attitude I may cite the fact of eggs found in the early stages of cleavage in the open harbor during the collection of material. This would certainly preclude the possibility of any osmotic, or other such extraneous influences as might become operative under the artificial conditions of the aquarium or laboratory. Moreover, it could hardly be possible that during a series of observations extending over at least three seasons the same phenomena should continually predominate unless more or less normal and constitutional.

Furthermore, additional experiments as to the influence of temperature go to show that while it may accelerate or retard the rate of development it has no perceptible effects on its superfical aspects or fundamental character.

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While experimental work in this connection on artificial parthenogenesis was not out of my thought, the difficulty of obtaining eggs in sufficient numbers for such observations precluded any attempt beyond a few simple experiments as to the effects of osmotic, or similar processes, the results of which were chiefly negative. It may be assumed, I believe, that whatever, of osmotic or other mechanical influences, might be involved they must be of only incidental or secondary importance, and in nowise disturb the general course of events.

In the earlier phases of cleavage there may be frequently exhibited more or less of regularity, especially during the first two or three cleavages. Following these it is however, difficult to distinguish the least trace of order or symmetry. While it may be possible to recognize something of general correspondence in the phases seen in a series of eggs, it would only be such as one might recognize in the phases exhibited by a series of amoebae of the same species during the course of an hour's activities. A comparison of the figures of the several plates will abundantly justify this statement. As a matter of fact these eggs during certain phases of development assumed shapes more or less comparable to amoebae, and were it important to establish a distinctive type of cleavage as characteristic of such eggs no more appropriate term than amoeboid could be proposed for it.

So far as I am aware nothing quite comparable with such a type has been hitherto described. METSCHNIKOFF ('86), has observed certain erratic features in the development of *Ratkea* and *Oceania*, but they differ in many points from that under consideration. WILSON ('84), has also recorded a considerable range of variation in the cleavage of *Renilla*, but was able to reduce it to some few predominant types. This it has been utterly impossible to do in the case of *Pennaria*, beyond the first few cleavages, as already pointed out. For these early phases there seem to be some two or three rather predominant features which it may be well to distinguish in this connection. First, what may be designated as a centripetal type, in which the direction of the first two or three cleavage furrows are toward the center of the egg, as shown in Figs. 22—26. Second, a type directly the opposite of the first, in which following the first cleavage the direction of the succeeding furrows was outward, or centrifugal, as shown in Figs. 8—9, 28.

Occasionally it was possible to distinguish a third tendency toward a rather vertical cleavage, tho of a very indefinite character, as seen in Figs. 37 and 38.

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Associated with these irregularities and adding in many cases to the anomalous and bizarre aspects of the eggs were the various ectosarcal features to which reference has been made in another connection. Except in some few cases no attempt has been made to represent these in the surface drawings of entire eggs. In Figs. 4, 15, 44 something of their optical aspects are shown.

It is not impossible that something of all these various irregularities may be due to the membraneless character of the eggs, tho I am inclined to ascribe it chiefly to an intrinsic amoeboid property of the protoplasm of the egg, a condition not lacking in other coelenterate eggs, notably those of *Hydra*. This condition during the early growth of the egg has already been referred to.

2. External aspects.

While in general an examination of the figures of the several plates will afford in some respects a more graphic conception of the superficial phenomena of cleavage than is possible from verbal description, still the latter cannot be wholly dispensed with, and the following descriptive notes may at least amplify and otherwise elucidate the features portrayed in the drawings, all of which were made from life by means of the camera lucida, including also the outline sketches of the text.

In Figs. 1—6 are shown a somewhat typical series of phases exhibited by an egg during early cleavage. Figs. 1—3 show traces of what might be called a cell lineage as far as the four cell stage, but this disappears in the following figures. This is also seen and may be traced somewhat more clearly in Figs. 7—11, but it is lost in Figs. 12 and 13.

On Plates XXIV, XXV, the figures show a somewhat more erratic aspect of the same general features. In Fig. 41, are seen the four cells, *A* to *D*, while in the following may be seen the next distinctive aspect, *B* and *D* have divided, the latter twice and almost equally, while *B* shows an indefinite cleavage. *A* and *C* remain unchanged. Beyond these features all lineal relations were lost as in the previously cited cases. An extremely erratic condition is shown in Figs. 14 and 15. As will be seen the pseudopodia-like blastomeres are almost wholly isolated from each other save for the bridge-like connectives of ectosarc. In Fig. 16 is seen an outline sketch of a section through an egg of similar aspects, tho of a later stage of cleavage. This will clearly show that such features are not merely

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amoeboid conditions of the surface protoplasm, but that they are actual expressions of internal cytological changes, involving nuclei as well as cytoplasm.

It not unfrequently happens that during the progress of cleavage, chiefly during its earlier stages, openings appear between the cells as shown in Figs. 57, 59, 60. These as frequently disappear, of course, and shade more or less gradually from one condition to the other, as the phantom blastomeres tumble promiscuously over each other in their multiform displacements and readjustments.

In other cases quite late in development, indeed with the approximate completion of cleavage, the embryo often assumes a dumb-bell shaped aspect closely resembling the egg in its two cell condition and impossible to distinguish from it except upon microscopic examination, in which condition it remains for considerable time, finally assuming the typical ovoid morula condition. Fig. 19 shows one of these specimens.

In the earlier paper attention was directed to the origin of two larvae from a single egg by spontaneous division at some period during cleavage. It would seem not improbable that it arises by such a constriction as here described passing on to complete fission. But whether produced by a fission at this time or some other, the fact of its occurrence is beyond doubt.

Attention was also called in the former account to another somewhat anomalous feature of cleavage, namely, the practically independent cleavage of the two halves of the egg following the first cleavage. It is unnecessary to reproduce the figure of this feature. In its place, and illustrating the same general aspect, is Fig. 16 showing the practically independent cleavage of a single portion of the egg connected to the main body by a narrow strand of protoplasm, and also the gradual cleavage of the larger mass from one side.

Other equally grotesque features were numerous enough, but those already cited will suffice to emphasize what was said in the opening paragraph of this section of our subject as to the anomalous aspects of the entire course of development.

3. Internal aspects.

Among the most significant of internal features, and naturally one first encountered in a study of sections for evidences of fertilization or very early cleavage conditions, is the fact that nuclear

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activity tends constantly to outrun that of the egg cytoplasm. This is well shown in Fig. 43, representing a stage just prior to the beginning of cleavage. Not to dwell upon the papilliform pellicles to which attention has already been directed, we notice the occurrence in the section of some five nuclear centers, four in the resting condition, the other in process of mitosis. A similar condition may also be seen by consulting various figures of the plates. As may be inferred from this putting of the case such a condition is not only not rare, but seems to be the rule. It is hardly possible to examine a series of sections of any fertilized egg which has approached the prophase of cleavage in which these conditions are not more or less evident. It would seem therefore that we have in *Pennaria* a condition not unlike certain found by WILSON in *Renilla*, and long known among crustacea, insects, etc. HICKSON ('93), has described a like condition in the development of *Distichopora*, and I have described in a forthcoming paper, the same condition in the development of *Eudendrium*.

It would seem therefore that we have here a case in which, as in others well known, there has been for some reason a break in the consonance or rhythm of the nucleus and cytoplasm, — that in some way not well understood, these cell organs have become measureably independent in their functional activities. Or should it prove true that there may occur such a condition as nuclear fragmentation and dispersal thru the cytoplasm, followed by a subsequent reorganization, as has been claimed by well known investigators, and as I have suggested in a previous section of this paper and in the paper on *Eudendrium*, we may have this as an explanation of the condition under consideration. Of the facts there can hardly be serious question. Whether their interpretation may be found in one or other of the methods suggested, or in some wholly different way, remains to be seen.

It should not be overlooked that this lack of synchronism in the activities of nuclei and cytoplasm continues in many cases throughout the entire history of development. In Fig. 45, which presents a later condition than that just previously cited there is shown unmistakeably the same condition, several nuclei appearing in a single blastomere, and within the limits of the same section. The same condition is likewise shown in Fig. 46. In Fig. 71 is shown a section through an egg at a comparatively late stage of development, indeed at approximately the completion of cleavage, the primitive ectoblast being

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fairly well differentiated over a considerable portion of the embryo, yet essentially the same features are exhibited as in the former figures. It may not be amiss to state that in none of these internal cellular bodies is there any cell membrane present. The limiting line in the figure serving only to indicate the more or less indefinite outlines of the several cells.

Again, it not unfrequently happens that eggs which during early development have shown a fairly regular and equal cleavage later show the same derangement of rhythm just referred to, the nuclei proliferating with great rapidity without any corresponding activities on the part of the cytoplasm. Indeed during later cleavage, and particularly after the assumption of the so-called morula condition, development seems to consist chiefly of nuclear proliferation associated with a coincident segregation of cytoplasm about each nucleus, which seems to act as a focal, or organizing center about which there occurs a sort of organic crystallization process. A study of the several figures cited in the preceding paragraph, together with others of the sectional series can hardly fail so suggest some such interpretation as that here proposed.

VIII. Comparisons.

Not a few of the features shown in the development of this embryo present more or less striking resemblances to those described by LILLIE, in the paper already cited, and but for the unmistakeable evidences of segmentation exhibited during the earlier stages of development one might almost adopt LILLIE's caption, »Differentiation without Cleavage«, as a fit designation of the phenomena.

Indeed as one works through the history of development as found in these eggs the entire flood of experimental observations which have been made upon cytology, from the classic and brilliant results obtained by the HERTWIG brothers ('87), on to the present time, seems to sweep in upon the vision. The effects of various narcotics, salts, mechanical, thermal and other extraneous agents are too well known to call for more than passing notice. LOEB ('96), NORMAN ('96), ZIEGLER ('98), MORGAN ('99), and a host of others have made noteworthy contributions along this line which have brought to light unthought of factors in developmental mechanics, as well as rendered obsolete others which had long been thought to be fundamental.

In his recent »Experimental Studies in Cytology« WILSON, ('01),

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has cited results, some of which have a more or less striking significance in comparison with the phenomena here under review. Under the head of »nuclear division unaccompanied by cytoplasmic cleavage« WILSON describes and figures conditions and features almost identical with many among the foregoing. The »dumb-bell-shaped« areas have something of a counterpart in similar figures previously cited, and the syncytial conditions found in several cases have much in common with those herein described, and with many known to exist among coelenterates.

Under the caption »progressive division of the centrosome without the multiplication of nuclei« he also describes the formation of »nucleus-nests«, a condition apparently analogous with similar nuclear conditions which I have described in the preceding pages.

The migration of nuclei toward the periphery of the egg, to cite another of WILSON's observations, is strikingly similar to that occurring in the eggs of many arthropods, and even more closely like those which I have described in the development of *Eudendrium*.

These comparisons might be extended still farther, and in many equally striking details, but the foregoing are sufficient to illustrate the general fact of the remarkable analogies afforded by many of these artificially obtained results as compared with those occurring in the natural development of not a few organisms belonging to these lower classes.

In reflecting upon such comparisons one is tempted to raise the question as to whether the analogies, so-called, may not be more correctly designated as homologies? If we are justified in regarding as homologous the remarkable resemblances discernible in the cell lineage of annelids, molluscs, etc., why not with equal justification so consider the almost equally striking resemblances exhibited by various eggs developing under the influences of changed conditions, or artificial agencies?

On the assumption that during the course of evolution embryological factors have been more or less profoundly modified to meet the constantly changing conditions of development, and that under special and crucial exigencies unusual adaptations may have arisen in the life history of certain forms, we might naturally expect to find occasional exhibitions of such embryogenic features. And such facts are indeed well known. In a recent paper on »Adaptation in Cleavage« LILLIE (op. cit.), has ably shown that just such embryonic adaptations manifest themselves, even in early cleavage. May it not

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have come to pass that at various points in embryonic evolution certain of the highly complex cytologic mechanisms have been gradually acquired to meet new conditions in embryogeny? It may be safely assumed that primitive protoplasm, or even primordial cells, did not possess such complicated structures as spindles, chromosomes, centrosomes, asters, etc. It may fairly be questioned whether among the simpler Protozoa or even among all tissues of certain higher classes these structures are essential, or invariable factors.

If, therefore, these assumptions and inferences are granted the further question arises, namely, whether the foregoing cytologic analogies, or possibly homologies, which have been passed in review, may not in reality be cytogenic recapitulations, so to speak, rather than freaks or abnormalities, as we have been wont to consider them? Of course, the question must, for the present at least, remain an open one. Only after long continued observation and experiments can we hope for a solution. When we remind ourselves, however, that in the outcome of these so-called abnormal processes of development normal embryos are obtained, and that apparently end-results of artificial methods in embryogeny are in many cases indistinguishable from those herein described, as well as in many others too well known to call for special mention at this time, which are confessedly normal at every step, the above queries and suggestions are really less preposterous or radical than might at first seem.

Of the facts involved there can hardly be serious doubt. Whether the interpretations proposed, or the inferences drawn, are justified or may prove acceptible may be quite a different matter. This is however, of less consequence than a clear appreciation of the facts themselves, which if accepted at their face value cannot be disregarded when the conditions justify a final attempt to formulate a general law of embryogeny.

IX. Ectoderm Formation.

In organisms having a fairly symmetrical and typical cleavage the problem of the formation of the ectoderm is comparatively simple. If the product of cleavage results in either blastophere or morula the peripheral cells are already in position and to some extent have the form of specific ectoderm. Where the segmentation is more or less unequal and the micromeres increase rapidly, overgrowing the macromeres, the ectoderm arises by the process known as epibole.

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In the case of *Pennaria* while there may be something in common with each of these processes in certain cases yet in many, probably most, there is little peculiar to either. In Fig. 72 may be seen in actual section an egg in which cleavage is approaching completion, tho with several macromeres yet unsegmented. These lie in part within the egg and in part at the periphery, while patches of fairly well-developed ectoderm are already established. With the progress of segmentation these macromeres are finally reduced to series of cells of symmetrical size, and during the process the further development of the ectoderm has gone forward at a corresponding rate, forming as shown in Fig. 73, a continuous peripheral layer over the entire embryo. It will only be necessary to glance at sectional figures of varying stages to perceive that the ectoderm is formed by a graduated process of cell differentiation and not by any direct derivation from cells of any specific character. The completion of the ectoderm may take place in many cases before that of the final resolution of the internal cells into a mass of uniform size, such as shown in Fig. 73. Indeed several hours may be involved in the process of this internal cell proliferation, often from six to ten, during which the only external evidence of the process is that of the gradual assumption of the pyriform shape characteristic of the planula.

During this time the cells of the ectoderm have taken on the more or less columnal form, later acquire cilia, and the hitherto quiescent embryo becomes a free-swimming planula, in which condition it continues for from four to ten days, the time varying as conditions may be more or less favorable.

It is no part of the present paper to follow the later details of ectodermal differentiation, such as the interstitial cells, cnidoblasts, etc. I may however merely note the fact that the mucus, or gland-cells, which constitute so remarkable a feature of the ectoderm of *Eudendrium* as this stage, are quite insignificant both as to number and size in the present planula.

X. Entoderm Formation.

In this phase of development *Pennaria* seems hardly less anomalous than in several of the preceding. It has generally been contended that among Hydromedusae the origin of the entoderm takes place through some two or three distinctive modes, namely, — First, Delamination, or a process of direct division of the early blasto-

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meres of the embryo in a plane more or less parallel with the surface, or in some cases a cleavage of the cells of the primitive ectoderm of the blastula in a similar plane, these inner cells by subsequent division giving rise directly to the entoderm.

Second, multipolar ingression of certain primitive ectoderm cells bodily into the cavity of the blastula, where they followed a process of direct entoderm formation as in the first case.

Third, unipolar ingression, or hypotrope, as designated by METSCHNIKOFF ('86, p. 70), in which there occurs a similar ingression as in the former case, but limited to a single pole of the blastula or planula. The same author has also designated several intermediate processes, such as primary, secondary, and mixed delamination. When, however, one undertakes to apply these distinctions in concrete cases it soon becomes more or less evident that at best they are chiefly conveniences for descriptive purposes and have little distinctive value as specific, or differential processes. Thus in such a case as that of *Pennaria*, for example, while certain embryos might seem to conform to one, and others to another of these modes of entoderm formation there are so large a number which conform to neither, but are unaccountably erratic from first to last, that to undertake to formulate any one, or even several, specific modes of formation would be as impossible as it would seem to be unnecessary. From a somewhat extended personal examination of cleavage among tubularian Hydromedusae, as well as in review of the more accessible literature of the subject it becomes more and more evident to my own mind that no one, or even several, specific modes of entoderm formation can be made to cover all cases.

In a forthcoming paper on the development of *Eudendrium* I have called attention to similar conditions, and likewise pointed out similar difficulties in the way of any specific mode of formation of the germ layers. Indeed in both *Eudendrium* and *Pennaria*, not to mention other cases, cleavage would seem to result primarily in the formation of a more or less characteristic syncytium, the subsequent development of the germ layers taking place by a gradual differentiation of the syncytial elements, first and naturally the ectoderm, and later, often very much later, the entoderm. In both these species a definite differentiation of entoderm is delayed till almost the period of metamorphosis, or during the entire planular history. In the meantime there has occurred a gradual reduction of many of the syncytial elements to a granular mass, including of course deutoplasmic

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granules, which serve as food during the larval period while the planula is entirely devoid of mouth or other means of taking food from without.

XI. Summary.

1) The eggs of *Pennaria tiarella* and *P. cavolini* arise in the ectoderm of the manubrium; they grow by the absorption of their fellows, those of *P. tiarella* engulfing them entire, while those of *P. cavolini* seem to dissolve them and absorb their contents in liquid form.

2) About the period of maturation the nucleus migrates to the outer periphery of the egg, the nuclear membrane dissolves, the chromatin seems to disintegrate and the entire nucleus is apparently dispersed throughout the cytoplasm. At any rate, its elements lose entirely their distinctive chromatophilous property, and remain for some time in this condition. Details of fertilization have not been determined.

3) Cleavage is total, but extremely irregular and erratic, hardly any two eggs showing closely similar phases beyond the first few.

4) About the period of fertilization and during early cleavage many eggs exhibit most interesting ectosarcal phenomena in the form of papillae, strands, etc.

5) During late cleavage the embryo often exhibits the aspects of a syncytium, internal nuclear proliferation going forward without definite cellular organization.

6) Entoderm formation follows none of the stereotyped forms, such as delamination, polar ingression, etc. It arises somewhat late in the planular life by a gradual differentiation of the indifferent entodermal cell mass.

7) Experiments upon egg fragments showed them quite capable of independent development, apparently whether nucleated or otherwise. Development of fragments was apparently normal in all respects, correspondingly small polyps resulting, tho with all the characters of ordinary polyps.

8) During early development the eggs often exhibit nuclear phenomena remarkably similar to well-known conditions induced by the action of narcotics, various salt solutions, etc.

Syracuse University, March 28, 1904.

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XII. Zusammenfassung.

1) Die Eier der *Pennaria tiarella* und *P. cavolini* entstehen in dem Ektoderm des Magens und wachsen durch das Einsaugen ihrer Gefährten. *P. tiarella* verschlingt sie vollständig, während *P. cavolini* sie aufzulösen und ihren Inhalt in der Form von Flüssigkeit einzusaugen scheinen.

2) Um die Zeit des Reifens wandert der Kern zu dem äußeren Rande des Eies, die Kernmembran löst sich auf, das Chromatin scheint sich aufzulösen, und der ganze Kern ist überall durch das Cytoplasma zerstreut. Wenigstens verlieren seine Elemente vollständig ihre Eigentümlichkeit sich stark zu färben und verhalten sich eine Zeitlang in dieser Weise. Das Detail der Befruchtung ist nicht bestimmt worden.

3) Die Furchung ist total, aber sehr unregelmäßig, ja regellos; wenige Eier zeigen ähnliche Phasen nach der ersten Furchung.

4) Um die Zeit der Befruchtung und während der frühen Furchung zeigen manche Eier sehr interessante ektosarcale Phänomene, so Papillen, Fäden usw.

5) In der späten Furchung zeigt der Embryo oft den Anblick des Syncytiums. Die Vermehrung der Kerne geht ohne bestimmte zellige Organisation vorwärts.

6) Die Entodermbildung folgt keiner der stereotypen Arten wie Delamination usw. Das Entoderm entsteht etwas später, in dem Leben der Planula durch eine stufenweise Differentiation der indifferenten entodermalen Zellmasse.

7) Die Experimente mit Fragmenten der Eier zeigten, daß sie der unabhängigen Entwicklung fähig seien, scheinbar kernlos oder nicht. Die Entwicklung der Fragmente scheint in jeder Beziehung normal zu sein, entsprechende kleine Polypen entstehen, und zwar mit all den Charakteristiken der gewöhnlichen Polypen.

8) Während der frühen Entwicklung zeigen oft die Eier Kern-Phänomene, welche außerordentlich ähnlich denen sind, wie sie unter den wohlbekannten Bedingungen der Wirkung von Narcoticis und verschiedenen Salzlösungen usw. entstehen.

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XIV. Explanation of the Figures.

Plate XXIV.

Figs. 1—6. Outline camera sketches of a single egg in cleavage, at intervals of 10 minutes. From life.

Figs. 7—13. Camera sketches, from life, of an egg showing fairly regular cleavage during earlier phases.

Figs. 14 and 15. Camera sketches from life of a very erratic cleavage.

Fig. 16. An egg in which a small portion *a*, segmented in apparent independence of the larger mass *b*, both portions later fusing into a single embryo.

Figs. 17 and 18. Two sections number 6 and 9 of a series of an egg, in which are shown the distinctively cellular character of the pseudopodia-like lobes. Internal cell-limits are devoid of membranes, the cytoplasm segregated in irregular cellular form about each nucleus.

Fig. 19. Section of a dumb-bell shaped egg, which before cutting had appearance of an egg in the two cell stage.

Plate XXV.

Figs. 20—26. Early cleavage phases of a single egg, drawn with camera from life. Figs. 22, 23, 25, showing aspects of centripetal cleavage. Figs. 24—26, with prominent ectosarcal bridge, or connective, at *c*.

Figs. 27—34. Cleavage phases of an egg drawn as before. Fig. 28 showing centrifugal cleavage. Figs. 29—32 showing connective ectosarcal strands.

Figs. 35—40. An egg showing irregular cleavage, limited largely to one pole. This egg is one of very few showing polar bodies during cleavage.

Plate XXVI.

Figs. 41 and 42. Two phases in a somewhat erratic cleavage. In the first is shown an irregular four-cell stage, the blastomeres *A B C D* arising directly from a typical two-cell stage. In Fig. 42 is shown the blastomeres *A* and *C* still undivided, while both *C* and *D* continue to divide irregularly.

Fig. 43. Section of an egg just prior to the beginning of cleavage, with ectosarcal papillae at one pole, near which may also be seen five nuclei. Fig. 43*a*. Section of similar egg showing cleavage beginning at one pole and gradually extending.

Fig. 44. Section of an egg during early cleavage, showing at one pole numerous papillae.

Fig. 45. Section of an egg during early cleavage, showing in several of the cells multinuclear conditions.

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Fig. 46. Section of another egg at a similar stage, showing likewise multinucleate cells. At *a* is shown a nest or chain of nuclear vesicles.

Fig. 47. Small segment of a section, drawn under ZEISS apochromat 2 mm showing apparently a maturation phase. The vacuolated condition of the cytoplasm in the area is quite evident, as is also the more homogeneous nuclear area near by.

Fig. 48. Small segment of another section of still another egg, drawn as before, in which are shown several nuclei in various conditions. Cf. the text, p. 462.

Figs. 49 and 50. Segments of similar sections of two other eggs exhibiting still other nuclear conditions. Cf. text, pp. 462, 463, 465.

Plate XXVII.

Figs. 51—61. Cleavage phases of a single egg, drawn from life, at intervals of from 5 to 10 minutes. Figs. 51—56 show the presence of papillae and strands, Figs. 56, 57, 60 show the flat, bridge-like films.

Plate XXVIII.

Fig. 62. An egg artificially divided in the two-cell stage, both blastomeres going forward in development without apparent interruption.

Figs. 63—69. Phases exhibited by blastomere *A* of last in progress of development.

Fig. 70. Section of an egg showing amoeboid aspects of form, and the internal conditions of nuclei and cytoplasm syncytium, indicative of the presence of currents or cytoplasmic movements.

Fig. 71. Section of an egg during late cleavage. Primitive ectoderm at *ec*.

Fig. 72. Solid embryo at completion of cleavage. *ec* the primitive ectoderm, *en* the entodermal mass.

Owing to the extremely irregular and inconstant shape of the eggs during cleavage no special attempt has been made to give exact measurements of magnification in sketches, except as specified in particular cases.

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Fig. 46.
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Fig. 47.
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Fig. 48
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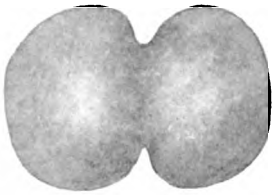

Fig. 20.

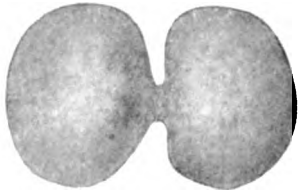

Fig. 21.

Fig. 22.

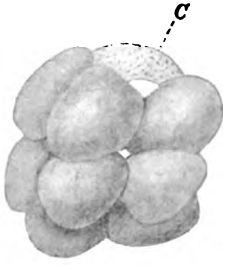

Fig. 26.

Fig. 27.

Fig. 28.

Fig. 32.

Fig. 33.

Fig. 33 a.

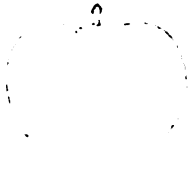

Fig. 37.

Fig. 38.

Fig. 39.

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Fig. 23.

Fig. 24.

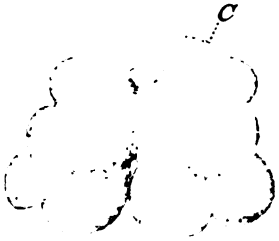

Fig. 25.

Fig. 29.

Fig. 30.

Fig. 31.

Fig. 34.

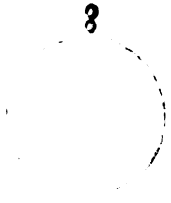

Fig. 35.

Fig. 36.

Fig. 40.

Engelmann in Leipzig.

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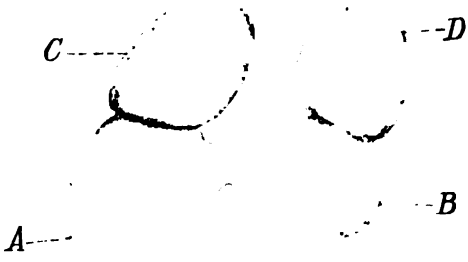

Fig. 41.

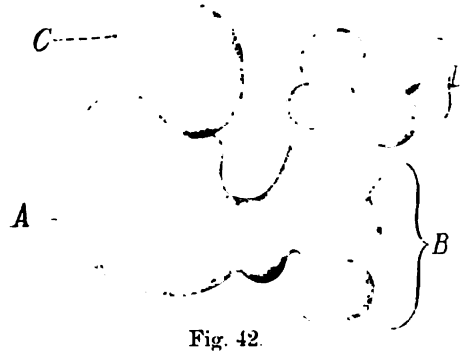

Fig. 42.

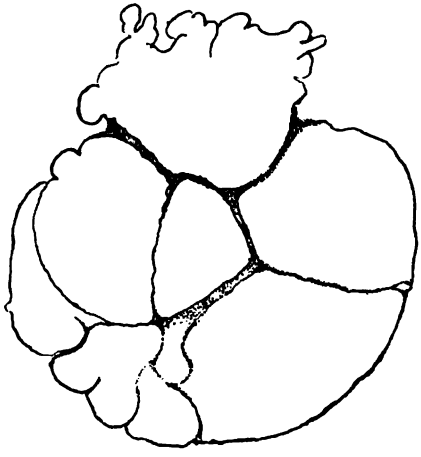

Fig. 44

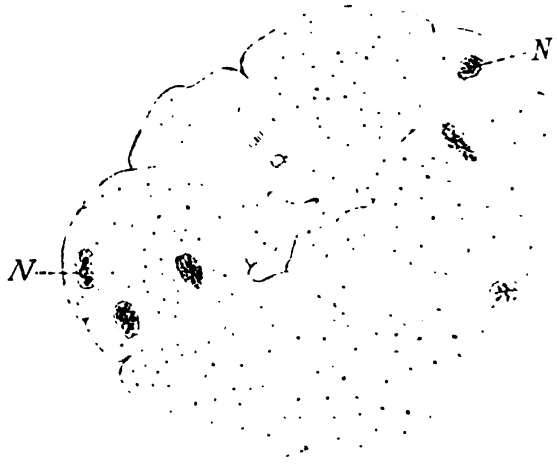

Fig. 45.

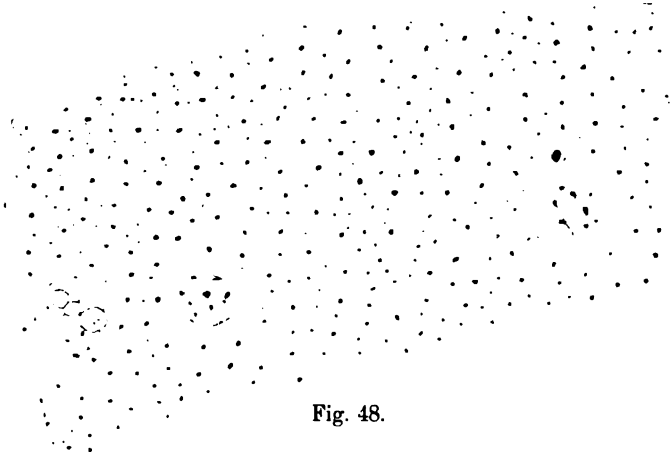

Fig. 48.

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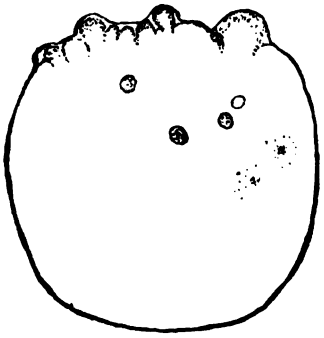


Fig. 43.

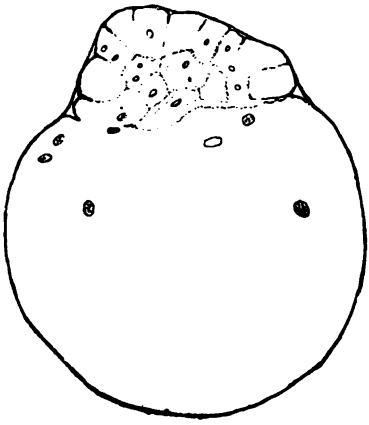


Fig. 43 a.

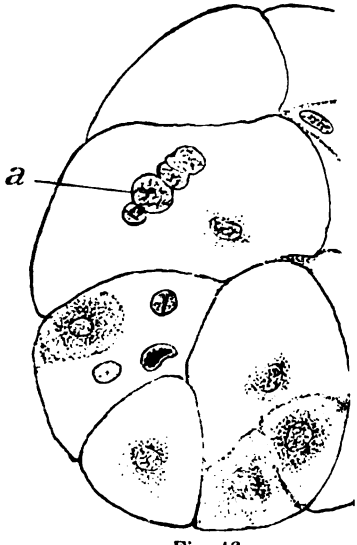


Fig. 46.

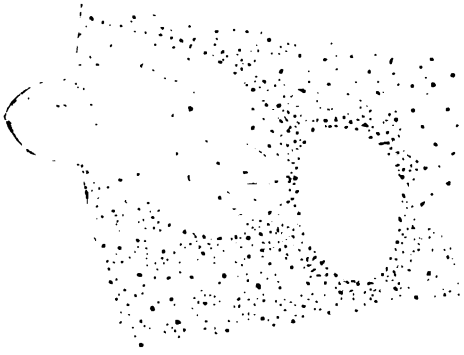
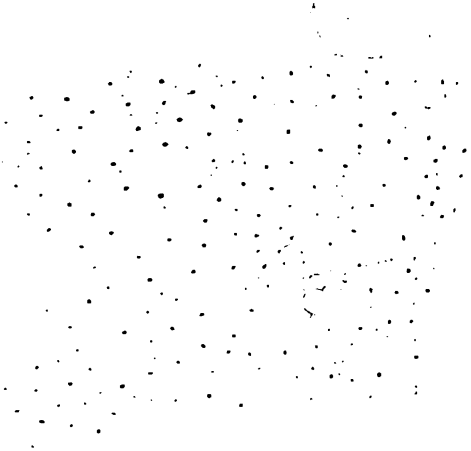


Fig. 47.



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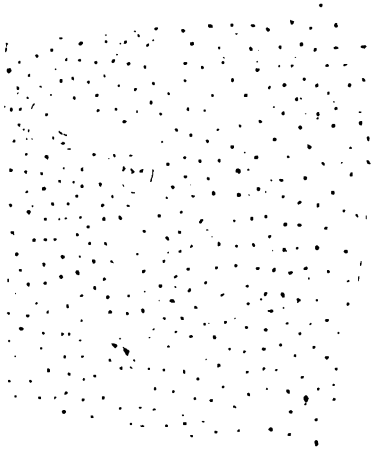

Fig. 50.

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Fig. 51.

Fig. 52.

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Fig. 58.

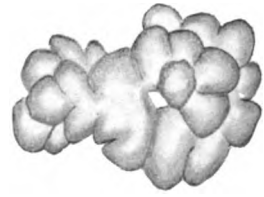

Fig. 59.

Fig. 60.

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Fig. 62.

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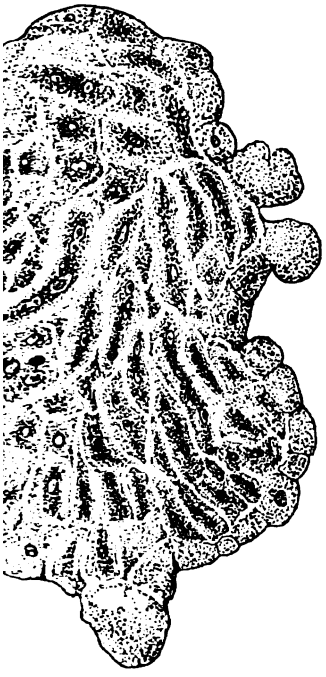

Fig. 70.

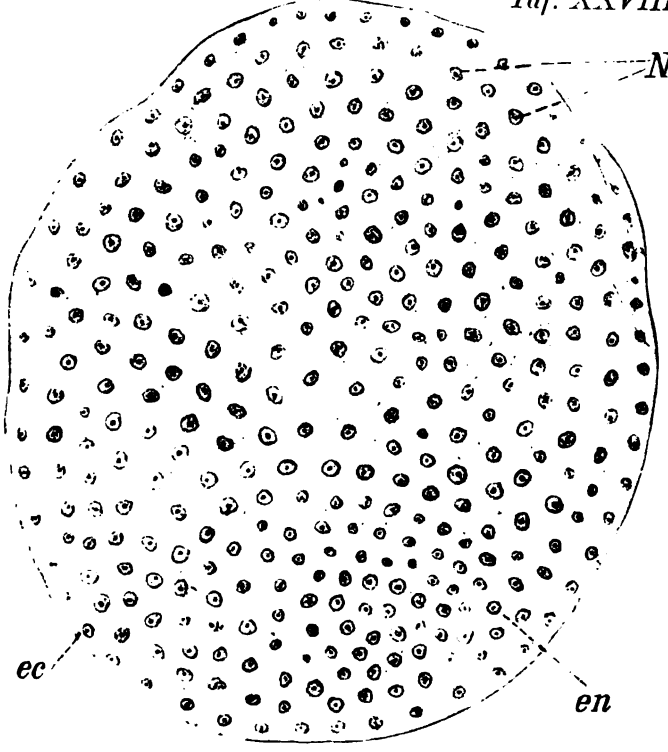

Fig. 72.

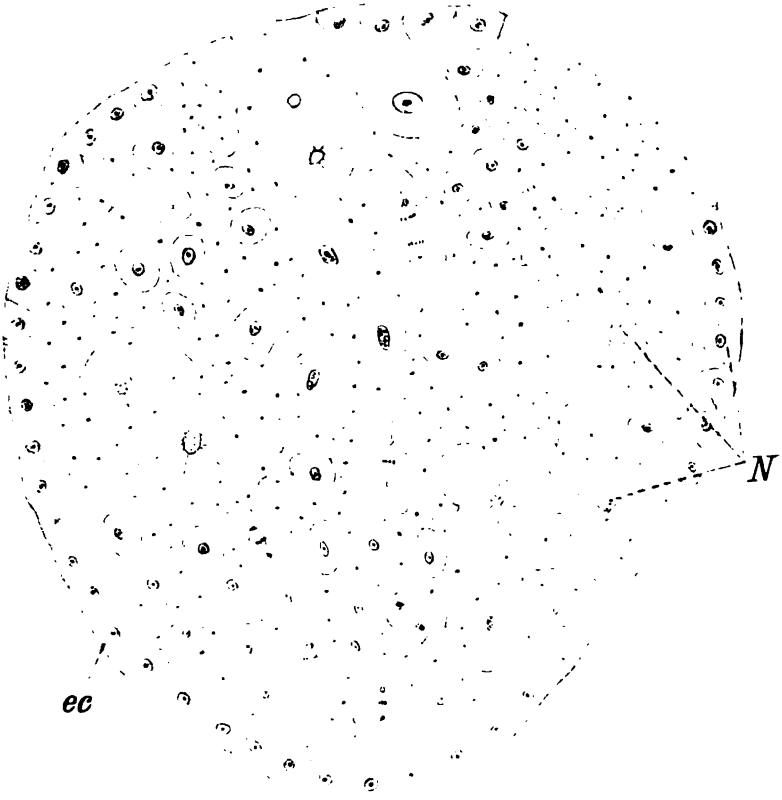

Fig. 71.

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(Mittheilungen a. d. zoologischen Station zu Neapel. 16. Bd. 4. Heft. 1904.)

Notes on some Hydromedusae from the Bay of Naples.

By

Chas. W. Hargitt

of Syracuse University.

With plates 21 and 22.

The following 'notes' on a few Hydromedusae obtained at Naples during the spring and summer of 1903 comprise a series of observations upon several species, some of which are believed to be new, others more or less rare, and all of more than ordinary biological interest. It is believed that the account submitted may have some value both as to the morphological and faunal facts concerned as well as a contribution toward a clearer knowledge of some hitherto disputed problems.

Pachycordyle Weismanni sp. nov.

During the month of May I obtained several colonies of an interesting hydroid which in many respects seems closely allied to *Pachycordyle napolitana* Weismann, but which will be seen to have some sharp differences. The colonies were obtained from the Bay of Naples at comparatively small depths and all inhabiting the shell of a living snail, *Fusus rostrata*. The hydroids live fairly well in the aquarium, having been kept under observation for as much as a week at a time. During this time several medusae were liberated, thus affording opportunity for critical study of the entire life history of the species, excepting that of the embryo. As intimated above, the hydroid seems to live 'fairly' well in the aquarium. It was, however, evident that with successive days under this changed environment there was more or less decline in vigor, both of the hydroid and developing medusae, for the first medusa, which was born during the first day in the aquarium, exhibited a vigor and activity

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quite lacking in those born several days later. The colony continued to live, however, and medusae were liberated during an entire week, at the end of which time the hydroids were killed and preserved for subsequent study of morphological details here-in-after described. Most of the material was fixed in alcoholic-corrosive solutions, and in 10 % solutions of formalin in sea-water.

It may be remarked, incidentally, that of the preserved material some was worked up soon after preparation, and other portions after my return several months later, and with an experience similar to that which I have elsewhere mentioned, namely, that better results were obtained from the newly prepared material than from that long preserved. This I believe may be regarded as a general rule having few exceptions so far as coelenterates are concerned. Sections were in almost all cases stained by means of HEIDENHAIN's iron-haematoxylin, followed in some cases by Bordeaux-red, with excellent results.

Systematic.

The genus *Pachycordyle* was instituted for a hydroid found by WEISMANN at Naples, and described by him in his monograph[1]. It is unnecessary to cite in detail his description of the hydroid except on certain points involved in comparisons.

As I have noted above, the general features of the present hydroid have much in common with that described by WEISMANN. The one feature in particular which would seem to sharply distinguish the two is the fact that WEISMANN's species is recorded as having only sessile gonophores while in the present case, as already cited, the gonophore is a free medusa, however, a short-lived one.

It should be noticed in this connection that WEISMANN's specimens were all male, while strangely enough in the present case I was only able to obtain female colonies. Whether this circumstance of itself affords room for doubt as to the specific difference of the two may be open to some question, of course, tho so far as my observations have gone, as a rule where the medusa is free in one sex of a given species it is almost invariably free in both. I have elsewhere shown[2] that occasionally specimens of *Pennaria tiarella* are found in which many of the female medusae discharge their

[1] Die Entstehung der Sexualzellen bei den Hydromedusen. Jena 1883 pag. 87.

[2] Amer. Natural. Vol. 34 1900 pag. 391.

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eggs without becoming free, tho I have not noticed this feature among male colonies.

It should also be said that in the species under consideration the gonophores in their earlier stages of development show every aspect, whether of form or structure, of sessile sporosacs. But since these come to maturity within a few days it would seem as if WEISMANN could hardly have failed to distinguish the development of the medusa and its probable birth. Moreover, he describes the gonophore as having a ramified spadix. While this may be the case during early development, in the mature medusa it has entirely disappeared. Whether here again the difference of sex may involve difference of structure must remain an open question, at least for the present.

Incidentally it may be worthy of remark that WEISMANN's specimens were obtained from considerable depths, '40 meters', and from the shell of a species of *Murex*, while as noted above the present specimens were all found on a shell of another genus and at small depths.

While owing to the fact that only one sex has been available for these comparisons and from which to formulate diagnostic definitions there may naturally remain some doubt as to the distinctness of the species, I am, however, strongly convinced of its specific independence, and venture to propose for it the name *Pachycordyle Weismanni*, in honor of the author of the genus and his distinguished contributions to hydroid morphology.

Specific Diagnosis.

Trophosome: Colony arising from a delicate, reticulated hydrorhiza. Hydrocaulus sparingly branched, from 3—8 mm high. Perisarc somewhat dense, not extending beyond the base of the hydranth, dull yellowish brown in color. Hydranths club-shaped, with sub-conical hypostome. Tentacles from 8—16, filliform, becoming delicate and thread-like when fully expanded (Pl. 21 Fig. 1).

Color: Hydranth body orange or reddish, hypostome whitish.

Habitat: Upon the shell of *Fusus rostratus*.

Gonosome: Medusa buds borne on side of stem, rarely on lateral branches, pear-shaped as they approach maturity, seldom more than two or three on a single polyp, the entire gonophore enclosed within a sheath of perisarc (gonangium?). The medusa escapes from the capsule by rupturing or dissolving the distal end, after which the

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capsule may often be found in a partially collapsed condition upon the stem or branches.

Medusa piriform, with prominent apical projection. Size 2 mm high by 1.3 mm broad. Velum narrow, velar opening very small. Manubrium rather large, conical and devoid of peduncle. Bell very transparent, with scattered nematocysts over the exumbrellar surface. Radial canals lacking, marginal canal simply a fissure of varying size between the layers of ectoderm with vestiges of entodermal lining near the margin. Tentacles entirely lacking. The medusa is devoid of color except on the manubrium, which varies from orange to dark brown. Mouth wholly lacking. When first liberated the medusa swims with a short, jerky motion, tho of limited vigor, a few contractions appearing to exhaust the little creature. Eggs are discharged almost immediately after the birth of the medusa, the life of which is very ephemeral, not exceeding one or two hours (Fig. 2).

Origin of the Germ Cells

Concerning the origin of the germ cells my observations upon this species agree in most points with those of WEISMANN on the related species, in so far as actual conditions are concerned. He finds these cells in the entoderm, and there only, yet rather insists upon the probability of their descent from ectoderm cells, while as yet in an indistinguishable stage. His specimens being all male in which, as he says, the primitive germ cells are small and correspondingly difficult of recognition, affords some plausibility for his contention, tho he likewise makes similar claims concerning the ultimate source of egg cells in other genera.

These speculative deductions are without any support from my own observations, tho as already noted, they were restricted to female colonies entirely and therefore cannot of course be claimed to prove those of WEISMANN to have been wholly in error. In the species under consideration the germ cells originate in the entoderm and are not found elsewhere at any time during their growth or maturation. As is very well known, the size and character of the egg cells render them easily distinguishable even at a comparatively early period in their development. In sections of the hydroid stem in both long and cross series and stained by various methods I have found no evidence of their occurrence other than in the entoderm.

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Moreover, these sections failed likewise to afford any evidence of WEISMANN's so-called "Keimzone". While eggs are found usually in greater numbers in the immediate region of the gonophore buds, as might be naturally expected, than in other portions, still they are found in not inconsiderable numbers at points more or less remote from this region, both above and below.

As a rule the egg cells are found in the deeper portions of the entoderm, tho not unusually they are found to occur likewise on the surface as shown in Fig. 3. Occurring in greater numbers in the region near the origin of the gonophore, and possibly also inciting its development, they seem to be carried into the bud during its formation and development. It would seem, however, that there is a migration of egg cells directly into the gonophore from adjacent portions of the stem, since it hardly seems probable that the large numbers which finally occupy the gonophore could have originated in immediate contact with it. Such a migration is well known in many other hydroids and may be accepted as occurring here, tho no direct evidence of it has been observed in the course of the present research. With the growth of the gonophore there seems to be a segregation of eggs into groups, or nests, about which there are developed follicular folds of the entoderm, as shown in Figs. 4, 5, in some cases entirely enclosing them. Others continue to lie in close contact with the entoderm lamella and finally form the superficial layer in the free medusa.

Development of the Gonophore.

As already intimated, the gonophore arises as a bud from the side of the stem, or more rarely upon a branch. It is at first indistinguishable from an ordinary hydranth bud, involving a direct evagination of both ectoderm and entoderm. The presence of eggs in immediate contact with it usually reveals its true character. From the first and throughout its entire development nematocysts are found in the ectoderm and multiplying during development furnish the nematocyst clusters of the exumbrella of the medusa. In iron haematoxylin preparations they stain a deep black during early development, as may be noted in several of the figures.

At first the gonophore is a simple club-shaped organ, as shown in several of the figures, but soon takes on a typical pear-shaped aspect, as shown in Fig. 5. At about this time there seems to be a cessation of the process of ovarian migration into it and very soon

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the proximal portions seem to constrict to form the pedicel of the gonophore. Somewhat later the constriction cuts off the gonophore from direct communication with the enteron of the stem, as shown in Fig. 6. Coincident with these changes there appear at the distal end of the organ, which up to this time has been simply a typical two-layered sporosac, a proliferation of ectoderm cells, the dissolution of the middle lamella and the ingrowth of the ectodermic plug to form the so-called bell-nucleus (Glockenkern), as shown in Fig. 4. The presence of numerous karyokinetic figures, as well as the micro-chemical reactions of the cells betoken the intense activity of the growing tissue. At first the bell-nucleus is rather globular in form. Very soon, however, it assumes an oval outline and continues to flatten and extend laterally in all directions. With this flattening of the structure there is reestablished the supporting lamella, a layer of entoderm is formed between it and the bell-nucleus, so that the latter comes to lie between the outer layer and the inner which contains the eggs. Coincident with this development and gradual extension of the bell-nucleus there has been a differentiation of its cells. Along its entire inner surface there has been differentiated a single layer of cells, forming in section a delicate chain-like series extending the entire length of the growing band, as shown in Fig. 5.

This growth and differentiation continues, gradually extending as a delicate sheet, laterally and proximally, the inner chain-like series forming an extremely delicate ectodermal membrane over the outer egg layer, the other layer of ectoderm forming the lining of the sub-umbrella of the medusa and overlying the thin layer of entoderm, to which reference was made above. This process continues till about half of the interior cavity of the gonophore is thus supplied with a double layer of ectoderm, one covering the eggs, which in the mean time have become somewhat evenly distributed over the developing manubrium, the other constituting the lining of the bell, as just mentioned, and as shown somewhat diagrammatically in Fig. 7.

With the completion of this ectodermic investment there occurs, apparently, both an expansion of the lateral walls of the gonophore to approximately the shape of the future medusa, and also a contraction of the entodermic walls into a more nearly cylindrical and conical shape, thus giving rise to the manubrium already referred to. This contraction to form the manubrium is apparently a purely

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mechanical process due, in part at least, to a reduction of the entodermic cellmass which has been involved in the transformation of a considerable portion of these epithelial cells into purely nutritive cells concerned in the growth of the eggs. Abundant evidence of this is found in the manifest signs of degenerative metabolism occurring throughout the entire mass. In many cases the cellular character had been lost entirely, in others it had been greatly modified. Figs. 4 and 7 show something of these changes.

With these phases of development completed the medusoid features may be said to be well established, though velum, tentacles, and canal systems are lacking. Only the first of these organs is finally developed. Tentacles are wholly absent; the bud-like projections shown at *m* in Fig. 7 are but sectional aspects of the bell margin, which as is shown in Fig. 2 of the fully developed medusa, is thick and rim-like. The velum is formed shortly before the birth of the medusa by a double fold of ectoderm, as shown in Fig. 7 *v*. This fold is apparently continuous over the entire velar end of the medusa, until shortly before birth, when a circular opening is formed by what seems to be an absorption of the central cells.

Of the canal system there is little to be said, since it is at best quite rudimentary. In Fig. 7 *c.c* is shown what in general position and form may be considered as the marginal canal. In no case have I been able to find traces of radial canals. In certain specimens sections would show a deeper fission of the entoderm layers at the junction of the apical projection and the lateral walls of the umbrella, and in others an extension of the marginal canal upwards, but there was no communication between the two. Furthermore, the manubrium is permanently mouthless, so that the medusa is apparently doomed to an ephemeral existence as an independent organism. And such is indeed the case. Of many specimens liberated from hydroid colonies in the aquarium none lived for more than an hour or two at most, as indicated in an earlier connection.

The apical projection is bulb-like, very thin and hollow as shown in Fig. 7. It is lined internally by an extremely delicate and tenuous layer of entoderm, the presence of which only becomes demonstrable under the highest powers of the microscope. The ectoderm layer over this region is likewise very thin, though more easily distinguished than the former.

As the medusae approach the period of liberation numerous orange-colored pigment granules appear in and among the entoderm

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cells of the manubrium, particularly in the median and terminal portions, giving to the loose tissue a distinctly brownish color. A careful study of the origin and development of these granules compels the conclusion that they are results of waste metabolism, or in other words, excretory products. When attention is directed to the fact that the entodermal epithelium of this region has been the center of active metabolism incident to the growth of the eggs from the time the gonophore bud became functionally disconnected from the body of the polyp, and that in their stages of growth these entoderm cells through cytomorphosis became essentially nutritive cells, the foregoing conclusion is only what might be naturally expected.

In connection with these observations it had occurred to me that possibly certain of these pigment granules might find their way into the growing eggs along with nutritive matter absorbed from the entoderm cells, but a careful examination failed to show that this was the case. Furthermore, since the pigmentation of the eggs occurs chiefly as they attain full growth, and coincident with the metabolism involved in the phases of maturation, I incline to believe that it is due in this case, as in the former, to pigment granules evolved through similar cytoplasmic metabolism. This view is strengthened by the fact that in the present case, as well as in others well known, the color of the eggs differs from that of the manubrium, to a greater or less extent.

In a recent contribution[1] I have directed attention to the relation of excretory processes to coloration among certain of the lower invertebrates, and the facts here cited afford additional evidence in support of the general proposition.

LENDENFELD[2], in a critical paper on *Eucopella* has briefly referred to the probable relation of the brownish pigment present in the radial canals of the medusa to excretory processes. He expressly says, "Diese Pigmentmassen gleichen denen, die in dem Entoderm der Nährthiere vorkommen, durchaus und glaube ich dieselben ebenfalls als Excretionsstoffe deuten zu sollen. Die Masse der Exkretionsstoffe richtet sich natürlich nach der Lebhaftigkeit des Stoffwechsels und es wird deshalb nicht Wunder nehmen, wenn gerade in den Radialkanälen der lebhaft wachsenden Medusenknospen sich solche Stoffe anhäufen."

[1] Science Vol. 19 1904 pag. 132.

[2] Zeit. Wiss. Z. 38. Bd. 1883 pag. 545.

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As will be seen from the foregoing account, we have here a medusa which is either extremely degenerate, or one of very primitive type. Its development within a chitinous capsule (gonangium?), associated with the sporosac-like history of the gonophore would seem to suggest the latter alternative. So far as I am aware the development of Anthomedusae within gonangia is quite rare if not wholly anomalous. The unique gonophores of *Dicoryne* are probably of very different character and significance.

In 1883 LENDENFELD (op. cit.) described a new hydroid and medusa, *Eucopella campanularia*, which apparently sustains a relation to the Campanularidae similar to that of the medusa herein described to the Tubularidae. The medusae of these forms have much in common, such as shape, size, rudimentary organs, etc.

Moreover, in the origin of the eggs, their migration into the gonangium which later gives rise to the medusae, the nutrition of the eggs and the general character of their later history, the correspondences between the two are very interesting. LENDENFELD's description of *Eucopella* also confirms the suggestion of a preceding paragraph that these are probably both generalized, or primitive types of medusae, in which the origin of the eggs, apart from, and prior to the organization, of the medusa, points to a condition similar to that found in such hydroids as *Hydractinia*, *Clava*, etc. in which there is nothing more than a sporosac within which the eggs develop after their origin in the body of the animal and later migration into these specialized nutritive capsules.

As the eggs approach maturity two rather marked changes are more or less apparent, one involving the character of the cytoplasm, the other that of the nucleus. In their earlier history the egg cytoplasm consists of a more or less viscid matrix through which is distributed a finely granular matter, the whole forming a somewhat homogeneous body. With the assumption of the spherical shape and typical size (about 0.9 mm), the cytoplasm becomes more predominantly granular in character and at the same time there appears in many cases a tendency toward vacuolation. I have elsewhere[1] called attention to a similar condition in the eggs of *Pennaria* at about this period in their history and the same thing has been noted by METSCHNIKOFF[2] and others. In *Pennaria* it had seemed

[1] Arch. Entwicklungsmech. 18. Bd. 1904 pag. 462.

[2] Embryologische Studien an Medusen. Wien 1886.

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to me to be associated with maturation and fertilization phenomena, but the latter feature must be eliminated in the present instance, since in the absence of male specimens it was obviously out of the question. Whether any of the immediate phases of maturation may have been involved is likewise open to doubt, since I was unable to demonstrate any of the ordinary features of this process, either in living eggs or in those sectioned and stained. Intimately associated with these changes were nuclear modifications of a more or less remarkable character. Prominent among them is the dissolution of the nuclear membrane which occurs shortly before the birth of the medusa and the discharge of the eggs. Following this there occurs a marked decrease in the mass of the nuclear substance, probably due to the loss of nuclear sap, or a dispersal of matter through the cytoplasm, so that the nucleus measures only about half that of the ovarian egg. Of still greater importance is the change which occurs in the chromatin network of the nucleus, which appears to wholly disintegrate and to disperse through the cytoplasm. Not the slightest trace of chromosomes or chromatin substance can be demonstrated in the nuclei or cytoplasm at the time of the liberation of the medusa. The nucleus itself, greatly reduced in size, may still be seen as a definite area of very homogeneous texture, but indefinitely merging into the surrounding cytoplasm, there being no trace of nuclear membrane.

Prior to these later nuclear changes is another involving the nucleolus, which during the earlier ovarian history is a body of conspicuous character and strong staining qualities. In sections of eggs of about the conditions shown in Fig. 8, or sometime prior to the complete dissolution of the nuclear membrane and perhaps inciting it, it is quite common to find nucleoli migrating bodily from the nucleus into the cytoplasm where they seem later to disintegrate and mingle with it. In several sections double nucleoli were found in a single nucleus, as shown in Figs. 8 and 9. In almost every case of this sort one of these bodies appeared appreciably smaller than the other, and often somewhat distorted in shape, as may be observed in the figures just cited.

Vacuolation of these nuclei was not an infrequent phenomenon. It usually consisted of one, or rarely two, slightly refractile vesicular bodies occupying an excentric position in the nucleolus. Careful search was made for evidence of directly dividing nucleoli, but without success. The occurrence of two in a given instance is probably

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due to the degeneration of the one and the formation of another de novo before the first had escaped from the nucleus.

Whether the phenomena herein considered lend further force to the previous suggestion that the vacuolation of the cytoplasm of the eggs was probably due to changes associated in some way with maturation, or whether they may not rather be interpreted as expressions of cytoplasmic activities, or phases of metabolism similar to those previously considered, may be somewhat problematical, and must so remain for the present, since my material is neither sufficiently varied nor abundant to justify further speculations or conclusions.

Tubularia mesembryanthemum Allm.

The conflicting accounts of the development of this hydroid which have been given by earlier investigators, particularly CIAMICIAN[1], METSCHNIKOFF (op. cit.), and BRAUER[2] led me to make some observations upon living specimens, and also to collect material from which to review certain of the problems involved, such as the mode of cleavage, formation of the germ layers, etc.

While from the living specimens I was able to follow the later history of the embryo, its final escape from the gonophore and some aspects of its independent life, it was not practicable to follow out with any degree of satisfaction the details of cleavage. This was done by means of sections of the gonophores made in several planes, supplemented in some measure by the preparation of entire gonophores stained and mounted as transparent objects, much as had been done in the case of living material.

The preserved material was submitted to one of my students, Miss HOWLAND, who under my direction cut and stained the sections and traced the phases of maturation and cleavage, and some of the aspects of the later embryo.

The differing accounts as to the origin of the germ cells given by CIAMICIAN and WEISMANN, and that of BRAUER seem to be rather a difference of interpretation than of fact. The germ cells have been found both in the ectoderm of the peduncle of the gonophore as BRAUER contends and in the ectoderm and entoderm of the spadix

[1] Zeit. Wiss. Z. 32. Bd. 1879 pag. 323.
[2] Ibid. 52. Bd. 1891 pag. 551.

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as had been maintained by CIAMICIAN and later confirmed by WEISMANN. I am inclined to regard both as true; or in other words, that the germ cells probably arise rather indiscriminately in both positions, though in some specimens rather predominantly in the one region and in others equally pronounced in the other region. As I have elsewhere shown this to be the case with species of *Eudendrium*, it is unnecessary to more than mention the matter in this connection. It would seem that in this species we have a hydroid in which the differentiation of the body cells of the two layers is physiologically less sharp than in others.

The growth of the egg is quite similar in most respects to that of other species of *Tubularia*, such as *larynx*, as described by DOFLEIN[1], and *crocea*, as described by ALLEN[2]. It has not been possible to confirm BRAUER'S view that those germ cells which are to give rise to eggs are sharply differentiated before they come into the gonophore, or during their migration thitherward. On the other hand the view of DOFLEIN that their impulse toward active egg development is the result largely of position and nutrition in the gonophore seems by far the more probable, and is in general agreement with similar facts in the case of other species.

Of the large number of germ cells which crowd the mature gonophore a comparatively few, perhaps three or four, assume the character of ova at any one period of development. Their growth results from the active absorption of their less fortunate fellows, as has been shown in many other cases, as by ALLEN and DOFLEIN, previously cited, and by the present writer in the case of *Pennaria* (op. cit.).

As in the case of most Tubularians, after the development and liberation of one series of embryos other of the primordial germ cells proceed to grow as the former had done, and thus the process continues for several generations. The case is very different, of course, in species with free medusae, like *Pennaria*. Here the eggs are all discharged at about the same time and the medusa dies shortly after; hence most of the germ cells are consumed in the growth of the first series of eggs. The same is probably true in most short-lived medusae, but with many others, successive generations of eggs are produced during the entire breeding season.

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[1] Zeit. Wiss. Z. 62. Bd. 1896 pag. 61.

[2] Biol. Bull. Chicago Vol. 1 1900 pag. 291.

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The differences as to the exact means by which the growing eggs appropriate the surrounding germ cells which characterize the accounts of CIAMICIAN, BRAUER, and DOFLEIN, seem to me to be similar to others already cited as to the region where they originate, etc., namely, a difference of interpretation and definition, rather than of fact. As I have shown in the case of *Pennaria tiarella* and *cavolini*[1], so it seems with species of *Tubularia*, there may be differences as to points of detail. In some cases the egg may engulf the germ cells bodily, later reducing them to a fluid condition; while in others this reduction appears to take place before absorption. But whether before or after, it must ultimately depend upon some process of reduction substantially digestive in character before final assimilation is accomplished. The only other alternative to the supposition is that of mere fusion of the protoplasm of the one with that of the other without any assimilative changes whatsoever. That this is not the case would seem to be proved by the fact of the persistence of these so-called pseudocells or nuclei within the eggs during the entire history of growth, and even that of development, as is well known by those who have concerned themselves with the subject. Apparently the disintegration, or reduction of these nuclei takes place much less rapidly than does that of the cytoplasm of the germ cells, so that they may be distinguished within vacuoles of the growing, or developing eggs long after their first incorporation.

Maturation and Fertilization.

Concerning these phenomena there is little to add to the earlier researches of CIAMICIAN and BRAUER. It has not been possible to confirm the observation of the former that during the process of maturation the eggs become more or less conical on the side from which the polar bodies are extruded. Since, however, his observations were made, in part at least, upon living eggs, while, upon this phase of the subject, my own observations have all been upon preserved material, there may be no definite contradiction. It would seem to me doubtful, however, whether any appreciable change of form could be observed upon eggs enclosed in fixed gonophores within which they are usually closely packed.

BRAUER's account is much fuller and more definite than is that

[1] Arch. Entwicklungsmech. 18. Bd. 1904 pag. 457.

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of CIAMICIAN. Concerning his accounts of maturation my own observations are mainly confirmatory. As to fertilization, however, I have not been able to obtain cytological details quite comparable with those described by him. Concerning nuclear phenomena associated with these processes my observations confirm in considerable degree those which I have elsewhere described in connection with the development of *Pennaria* and *Eudendrium*. There is the same migration of the nucleus to the periphery of the egg, the dissolution of the nuclear membrane, and the same apparent dissipation of the nuclear substance throughout the cytoplasm of the egg, followed later by its apparent reorganization just prior to the first signs of cleavage.

Cleavage.

It is perhaps concerning this phase of the life history that most dissension has arisen. CIAMICIAN was the first to critically study its development, and in doing so directed his observations largely to the aspects presented by the living eggs. This he did by cutting off clusters of gonophores and studying them under the microscope. But as he confesses, the various contractions of the several parts of the gonophores and the resulting movements and readjustments so shift the position of the eggs that it is extremely difficult to obtain accurate observations.

He concluded that cleavage was unequal, the first and third furrows being at right angles to each other and meridional, the second equatorial and from within outward. In later cleavages there is a more rapid division of the cells at the animal pole, these later growing over the blastomeres of the vegetative pole by a process of epibole and enclosing the latter which later form the entoderm.

This conclusion has been frequently called in question by later observers. BALFOUR[1] claimed that an examination of segmentation by means of sections failed entirely to confirm CIAMICIAN'S results. CONN[2] has likewise failed to find any evidences of epibolic gastrulation in another species of *Tubularia*, and from this has undertaken to discredit CIAMICIAN'S conclusions. As is well known from later observations different species at times show very considerable va-

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[1] Comparative Embryology, 2d Edition, 1885, Vol. 1 pag. 154.

[2] Z. Anz. 5. Jahrg. 1882 pag. 483.

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riation in matters of cleavage, particularly among hydroids, and CONN's contention is not therefore, valid as an objection.

The researches of BRAUER, however, leave little doubt that on this point CIAMICIAN was evidently mistaken, and my own observations tend to confirm this conclusion. BRAUER maintains that cleavage in this species takes place by two sharply different processes, namely, first, by a more or less regular mode of fairly equal cleavages during the two meridional furrows, later ones being more or less unequal and irregular. In the second mode cleavage involves the nuclei only, which proliferate independent of the cytoplasm, forming a multinucleate egg, after which there follows a cleavage of the egg into as many cells as there are nuclei. In this process there may be considerable variety. It may occur when there are but few nuclei, four or six, or it may not begin until there are sixteen, or perhaps more.

My own observations upon this point, while confirming in a measure those of BRAUER, do not warrant any such sharp conclusion as to the distinctness of these processes. One may find a regular cleavage into two and four cells, etc., and he may also find an internal nuclear proliferation, in some cases indeed, many more nuclei arising than BRAUER has indicated, before cell organization and cleavage take place. But I have not been able to sharply differentiate these modes. They often graduate imperceptibly into each other, indeed, may be found occurring in the same egg, though this is not common. I am inclined therefore, to consider them as only varying phases of cleavage conditions more or less common among Coelenterates, particularly among Hydromedusae. This is notably the case with *Pennaria*, as I have recently shown, and as ALLEN (op. cit.) has shown in the case of *Tubularia crocea*.

As illustrating somewhat more clearly than is possible by merely verbal description I have included a few sketches made by Miss HOWLAND in connection with a study of sections already referred to.

In Fig. 9 is shown an egg in which nuclear proliferation is under way, but in which no signs of cleavage are present. This condition may continue somewhat indefinitely, as already suggested, till finally a syncytium is formed, very much as in the case of *Pennaria* (op. cit.), and as shown in Fig. 11, from which by a graduated process of cell organization and differentiation the germ layers are finally established, as shown in a later paragraph.

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In Fig. 10, is shown a section of another egg in which likewise the nuclear conditions are very similar to those of Fig. 9, but in the present case evidences of cleavage are present as shown at regions marked *c.pl.* Here there is apparently no close relation between nuclear activity and that of the cytoplasm. This again is quite in keeping with conditions well known in other cases.

In Figs. 12—23 are shown a series of simply outline sketches illustrating the striking irregularity of cleavage in a large proportion of these eggs. Figs. 12—17 (Pl. 21) show the first six sections in a series of twelve of a single egg. The sections begin evidently at the animal pole, if we may assume polarity in these eggs, as only in the first four sections are nuclei present, the later sections showing only the large and very unequal blastomeres of the vegetal pole. In Figs. 18—23 (Pl. 22) are shown likewise a similar series of another egg, but in this case the sections are vertical instead of horizontal, as in the previous case. In this case the six sections are the central ones of a series of fourteen, and show the gradual progression of cleavage from the animal pole, the other remaining undivided.

It is probably from observations upon similar cleavage phenomena that CIAMICIAN conceived the presence of an epibolic gastrulation, an interpretation quite likely to be made where surface views chiefly are under consideration. But as shown in sectional series it is just as evident that BALFOUR'S contention against such a process is indisputable.

Formation of the Germ Layers.

Concerning the origin and formation of the germ layers in this and related species views have been hardly less conflicting than on points already considered.

According to CIAMICIAN, as previously suggested, an epibolic gastrula is formed. This seems so obviously improbable, and moreover, so lacking in evidence as to demand little attempt at refutation. It is not difficult to recognize conditions in cleavage which might easily be construed from superficial aspects as comparable with this form of gastrulation, but many hundreds of sections of eggs in all stages of development have not afforded the slightest evidence of such a process.

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According to METSCHNIKOFF[1] a true morula is formed. 'The cells of the morula appear to be identical in structure and consequently to show no difference between ectoderm and entoderm. Only after the number of embryonal cells has become quite considerable do the elements lying at the periphery form a connected layer, which is differentiated from the inner parenchyma mass by a sharp contour.'

BRAUER maintains that cleavage results in a coeloblastula, the entoderm arising by multipolar delamination from the cells of the blastula. While the embryo at this stage appears morula-like, he maintains that it is really a bilamellar embryo, with both germ layers established, though not yet sharply differentiated.

So far as my own observations go they tend to confirm the conclusions of METSCHNIKOFF, though in some degree agreeing also with phases of BRAUER's conclusions. I have found no evidence in support of the view that the germ layers are established during early cleavage, as BRAUER has claimed, nor that they result chiefly from a delamination of any sort, whether primary or secondary, unipolar or multipolar. That a morula-like condition often results from the more regular type of cleavage there can hardly be serious doubt. On the other hand it is often just as evident that a syncytium is formed, especially in cases of nuclear proliferation. Here the differentiation of the germ layers is brought about by a slow and graduated process of cell organization and adjustment. The conditions found in this species resemble in many respects those which I have described in *Eudendrium* and *Pennaria*. For a considerable time after the ectoderm is fairly established, the entodermal mass continues as a more or less homogeneous aggregation of cells in an undifferentiated condition. Finally the cells adjacent to the ectodermal lamella assume a more or less columnar shape, form a continuous series, and thus give origin to a definitive entoderm. Coincident with these changes, or shortly following, the inner mass of cells show signs of disintegration, become vacuolated and tend to liquefy, thus giving rise to the gastric cavity of the embryo.

Later History of the Embryo.

Concerning the formation of the actinula there is little to be added to the account given by CIAMICIAN. The embryo becomes

[1] Embryolog. Studien an Medusen 1886 pag. 69.

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more disk-shaped, tentacles bud from the margins of the disk, and continue to grow in length, the embryo becomes entirely free in the cavity of the gonophore and later escapes as a typical actinula, differing in no essential respects from those of other well known species of *Tubularia*.

The actinula is not ciliated and has therefore no special organs of locomotion. On escape it is wholly at the mercy of the waves, or currents for distribution. In the aquarium it usually sinks close by the parent colony, often indeed falling among them, and seems to fix itself promptly upon any object with which it comes in contact, even the stems of parent hydroids. A careful inspection of colonies of hydroids which were discharging actinulae in the aquarium revealed the presence of young polyps in various stages of growth, from those apparently but recently attached, to specimens of considerable size, and of such maturity that gonophore buds were beginning to develop. It was not unusual to find several of these attached to a single stem, as shown in figure 24 (Pl. 22). As will be noted these young polyps show a definitely annulated perisarc over the entire length of the stem, whereas in the adult condition the perisarc is wholly devoid of this feature.

These observations recalled others of a similar character made upon *Corymorpha pendula*, a brief account of which I have elsewhere recorded[1]. There were in these cases conditions which seemed to indicate the probability that the small polyps attached to the bases of the hydroid might be independent organisms, and if so perhaps a new species, and they were provisionally so designated, under the name of *T. parasitica*, as indicative of their presumed relation to the larger organism. It is unnecessary to go over in detail the grounds which seemed to justify this conclusion. Suffice it to say that the total annulation of the perisarc of the small polyps, the deeply insinuated rhizoidal attachment, and the presence of gonads were among the more obvious and apparently distinctive characters, which seemed to point toward their specific independence.

The observations above recorded concerning the history of the polyp of *T. mesembryanthemum*, and also a brief note by Torrey[2] concerning similar observations on the young of *Corymorpha palma* incline me to reconsider my earlier views concerning the case of

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[1] Amer. Natural. Vol. 36 1902 pag. 549.

[2] Hydroids of Pacific Coast. in: Publ. Univ. California Z. Vol. 1 1902 pag. 38.

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C. pendula. In so doing, however, it should be noted that the attachment of the polyps in the two cases is very different. In the case of *C. pendula* there is a deep insinuation of the finger-like roots within the perisarc of the base of the hydroid, and usually low among the rhizoidal filaments of the adults, while in the case of *T. mesembryanthemum* the attachment of the young is purely superficial, and occurs at any place upon the stem.

Perigonimus napolitanus sp. nov. (?)

Of the twelve known species of *Perigonimus* but two have been recorded from the Bay of Naples, namely, *P. linearis* Alder, by Du Plessis[1] in 1880, and *P. cidaritis* Weismann[2], in 1883. Weismann has contended that Du Plessis was mistaken in ascribing his species to *P. linearis*, believing it to be that which he has described as new under the last of the above named species.

During two different times, in 1894 and again in 1903, I have sought to identify one or both these species among the Naples hydroid fauna but have not succeeded. A hydroid taken from the same habitat, namely, the spines of the sea urchin, *Dorocidaris papillata*, in 1894 and at first thought to be the species recorded by Du Plessis, was found to lack characters distinctive of the species as described by Alder and moreover lacked also characters enumerated by Weismann, particularly as to size, number of tentacles, etc. both of the hydroid and medusa. Weismann's species has been described as 9 cm high and to have 20 tentacles. The medusa differs from those of most species in having 4 marginal tentacles, and well developed oral tentacles with tufts of nettling organs.

Hartlaub[3] has called attention to the unusual characters of Weismann's medusa and has suggested that they might reasonably be assigned to a different genus, or even to a different family, so far as their fundamental characteristics are concerned.

When, in addition to this, one reflects that hydroids of commensal habits, such as is the case with most or all the species of *Perigonimus*, are usually of small size, and when furthermore it is

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[1] Mitth. Z. Stat. Neapel 2. Bd. 1880 pag. 146.

[2] Entstehung d. Sexualzellen bei d. Hydromedusen. Jena 1883 pag. 117.

[3] Zeit. Wiss. Z. 61. Bd. 1896 pag. 161.

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recalled that WEISMANN describes the perisarc of *P. cidaritis* as only a thin, slimy covering, the height of the slender stemmed hydroid, 9 cm, is of itself most remarkable. So far as I am aware, no species yet described has a height of more than about 8 mm, except *P. muscoides*, in which the height is doubtfully given as 2 to 3 inches.

Concerning the characters and affinities of *P. linearis* there has always been more or less doubt. It was originally described by ALDER as *Atractylis linearis*, and was later referred by ALLMAN[1] to the genus *Perigonimus*, but with a confession of grave doubt as to its real affinities, not having himself seen the species. I have previously expressed some doubt as to the occurrence of this species at Naples. Specimens from the collections preserved at the Zoological Station and marked *P. linearis*(?), have proven to be quite a different hydroid. And though I have sought during two summers, as intimated above, to secure the species it has not been found.

Upon the two different occasions previously cited I have obtained a hydroid which is undoubtedly a species of *Perigonimus*, but have not been able to identify it with either of the species above mentioned, nor with any other hitherto described. It was taken first in June, 1894, and again in April, May and June, 1903. In both cases the hydroid colonies were vigorous and large, but no gonads were present, a fact which would seem to suggest that the breeding season had probably past, or perhaps had not yet arrived. WEISMANN found his species in March, at which time gonophores were present. My own observations cover a period from March 6 to July 30, so that it would seem strange not to find some colonies bearing gonophores, yet such have been the facts in the present case.

In both instances the habitat of the colonies conforms well with that of members of the genus generally, namely, a commensal one. The hydroids were found in a few cases upon the shell of a shallow water gasteropod, but in most abundance and in best condition upon the margin of the carapace and upon the legs of a common crab, *Carcinus maenas*. It is an interesting coincidence that the species which it most resembles in general aspects, *P. jonesii*, of Long Island, N. Y., has a similar habitat on the spider crab, *Libinia emarginata*. This commensal habit of *Perigonimus* is a most interesting one, though not peculiar, since it is shared by a considerable number

[1] Monogr. Gymnoblastic Hydroids. London 1871 pag. 329.

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of well known cases, such as *Hydractinia*, *Podocoryne*, etc. But in *Perigonimus*, a genus comprising perhaps the largest number of species among Gymnoblasteae, so far as detailed information is available it seems that almost without exception the species are essentially commensal.

In common also with most known species the one here under consideration has a gelatinous perisarc, which covers the entire colony from the hydrorhiza to the body of the hydranths, and covering the latter to the bases of the tentacles, though not in any appreciable degree extending to the tentacles, as seems to have been the case with WEISMANN'S species. There can hardly be reasonable doubt that the mucus-like covering is a definite secretion by the hydroid itself, and homologous with the chitinous perisarc common to hydroids generally.

A character in the present species is the long, slender, stem-like branches which arise at sharp angles from the stems and grow quite long without signs of giving rise to hydranths. There seems to be evidence that eventually hydranths arise from their tips, but it is not conclusive, as in only a few cases have newly formed hydranths appeared upon them.

The following are the more distinctive diagnostic characters of the species, which seems to be new, and for which the name *P. napolitanus* is proposed: —

Trophosome (Pl. 22 Fig. 25): Hydrocaulus composed of numerous stems, variously branched, some of the latter without hydranths, all branches arising at sharp angles from the main stems or branches, entire hydrocaulus arising from a net-like hydrorhiza, and attaining a height of from 4—8 mm. Hydranths elongate, more or less fusiform, with conical hypostome, and with a single whorl of rather stout tentacles, 7—12 in number. Entire colony provided with an indefinite, gelatinous perisarc, extending over bodies of hydranths to close beneath the circle of tentacles. Perisarc sometimes dense and somewhat chitinous in older portion of stems and hydrorhiza, and of dull brownish color. Gelatinous perisarc often studded with foreign particles, diatoms, etc., which thus give to the colony a rough, or fuzzy appearance, as shown in fig. 25.

Gonosome: wholly unknown.

It is worthy of mention in this connection also, that I took on May 4. 1903, a hydroid which, barring the absence of gonophores, bore a close relation to *P. repens* Wright, and is probably identical

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with that species. As in the former species, and as just mentioned, no gonophores were present, rendering impossible full determination of the identity suggested. A careful comparison of the hydroid with the descriptions and figures of HINCKS leaves little doubt in my own mind, and if later observations of the species when the medusae are present shall confirm this view it will extend the range of distribution of the species to Mediterranean waters, as CALKINS[1] report of it in 'Hydroids from Puget Sound', has extended its distribution to Pacific waters.

Gemmaria implexa Alder.

In 1880 DU PLESSIS (op. cit.), records the occurrence of the medusa of this species at Naples during the month of March, and makes the very interesting remark that the specimens taken differed from typical medusae of the species in having 4 complete marginal tentacles instead of 2. »Encore cette méduse diffère-t-elle par quelques points de celle qui a servi de type aux descriptions qui existent dans les ouvrages anglais. Ainsi cette méduse présente 4 tentacules marginaux complets au lieu de deux seuls comme l'indique ALLMAN«.

DU PLESSIS makes no reference to the hydroid and presumably he had only the medusa taken in the 'Auftrieb', which leaves the question of identity an open one, at least in part, and particularly so in that his medusa had the unusual number of four tentacles.

On May 13, I obtained a small colony of the hydroid from which in a short time after having placed it in the aquarium a medusa was liberated, and within the following day several others. During the month I obtained about a dozen additional colonies of hydroids and placing them in separate aquaria obtained from most of them free medusae, which lived apparently quite normally for nearly a fortnight, thus affording ample time for critical observations as to growth, habits, etc. In Pl. 22 Fig. 27 is shown a sketch of the entire colony, and in figs. 28, 29, are shown also sketches of individual hydranth, and medusa.

As to habitat all the colonies obtained by me were found upon the live shells of small lamellibranch molluscs, apparently belonging to the genera *Nucula* and *Corbula*. According to HINCKS and ALL-

[1] Proc. Boston Soc. N. H. Vol. 28 1899.

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MAN the habitat varies from tide pools to considerable depths, and on fragments of rocks, shells, etc.

While conforming in most respects to the diagnoses given of the species by HINCKS and ALLMAN (op. cit.) in some few points there are differences, though not to my mind of any considerable importance. As to size HINCKS gives the height of the hydroid as from 12 to 20 mm, while according to ALLMAN it averages about 6 mm. In my own specimens the height varied from 3 to 6 mm in the expanded condition. It would seem that HINCKS must have had very unusual specimens, or that in some way his figures are incorrect.

A point of most difference is that concerning the location of the gonophores on the hydranth. According to both the previously named observers these bodies arise low upon the body of the hydranth and in a single, or sub-verticillate cluster. I have found them arising in several clusters or even singly, at any point or portion of the hydranth, and quite as frequently near the oral as the basal region of the body. They are borne on short pedicels, frequently in clusters of from two to four on the same peduncle.

The Medusa. At birth the medusae are sub-spherical and average about 0.6 mm in diameter, and have two well developed tentacles, which arise from large bulbous bases on opposite sides of the body, each tentacle provided with numerous stalked nematocysts. As noticed in a previous paragraph, they live well in the aquarium, but do not appear to grow to any appreciable extent. The largest specimen measured 0.8 mm in height by 0.7 mm in breadth. The general aspects of the little creature are shown in fig. 29, but no figure can delineate the elegant grace and beauty which it exhibits when floating languidly with tentacles extended, like delicate streamers fringed with an exquisite embroidery of stalked batteries of nematocysts, to a length of ten times the diameter of the bell, or quivers in magic undulations as the limpid creature pulses itself through the water. Little wonder is it that, observing something of its exquisite features that prince of earlier observers, MCCRADY, as expressing his sense of the beautiful, ascribed to the genus the name *Gemmaria*, the little gem!

As ALLMAN has well remarked, one of the most unique among the several characters of this medusa is that of the numerous, stalked capsules of nematocysts which fringe the tentacles. From near the basal bulbs to their very tips, the tentacles are irregularly covered

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with these interesting organs. To this author's graphic description of them (op. cit. p. 225), it is unnecessary to add. In but few particulars do my own observations differ in any material respect from his. In the first place my specimens have not shown the abrupt ends of the tentacles which he has figured. As will be noticed from the figure already cited, the tentacles usually have attenuated distal portions as shown. Again the body of the tentacles is more or less roughened or rugose, due to the irregular projections of the ectoderm cells in pseudopod-like processes. And finally I have not been able to observe the peculiar vibratory shimmerings of the pedunculated nematocyst capsules to which he has referred. In repeated observations directed to the detection of just this phenomenon I have as often failed to distinguish it. Possibly it is only an occasional phenomenon, arising perhaps under special conditions which may not have been present in the case of my specimens, or possibly it may be lacking entirely in some cases. I am in full accord with ALLMAN as to the essentially 'sarcodal' nature of the peduncles of the nettling organs, as well as to their irritability and contractility. It seems to me that the rugose appearance of the tentacles is likewise due in part to essentially the same ectosarcal activity of the cells of the ectoderm, which seem to thrust out and retract pseudopod-like processes of varying shapes and sizes and in part to the nettling organs with which they are studded. Vesicular cavities in the entoderm of the tentacles are frequently to be seen, within which are exhibited exquisite displays of Brownian activity among minute pigmentary granules. The same phenomena also occur in the irregularities of the walls of the radial canals.

As will be seen in figure 29, there is an oval capsular enlargement arising at each perradius of the margin just over the radial canal, containing large nematocysts, the cnidocils of which may be seen as bristling projections from the outer surface. Usually those of the perradii bearing the tentacles are larger than those of the others.

The medusae are very transparent except for the brownish color of the manubrium, the delicate brownish pigment of the radial canals, and the reddish orange pigment of the basal bulbs of the tentacles.

The earlier reference herein to the medusae of this species observed by DU PLESSIS, which seemed peculiar in having four marginal tentacles, recalls the fact that the medusa originally described

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by McCrady[1] and for which he established the genus, had likewise four tentacles. In 1900 Mayer[2] obtained a hydroid at the Tortugas which he described as the trophosome of the species, *G. gemmosa*, the medusae of which when first liberated had but two tentacles, nor did others appear.

The question naturally arises, whether in these cases we may have a different species, or whether the medusae of the same species occasionally vary as to the number of tentacles. The latter seems to me the more probable alternative. It is well known that medusae of different species, or even of different genera, often resemble each other so closely as to appear to be identical. Hartlaub, for instance, as cited in a previous connection, has shown this to be the case in several instances which need not be reviewed in this connection. Such is apparently the case also with the medusae of *Gemmaria* and *Corynitis*. I am strongly convinced, however, that in this case we have to do, not with two distinct genera, but with different species of the same genus; for it has never seemed to me that there are differences between the two which are sufficiently marked to justify generic distinction. And what is true in this case is probably true in many others of similar sort. This is doubtless due in large measure to the fact of the separate and independent life of the two organisms, hydroid and medusa. When we shall have worked out critically the ontogeny of the several species, much of the apparent anomaly will doubtless disappear.

Corydendrium parasiticum Cavolini.

Among the many species of hydromedusae which have long engaged the interest and observations of naturalists, few if any, surpass the one here under review. It was originally described by Cavolini[3] in 1785 under the name of *Sertularia parasitica* in a small monograph upon the Naples fauna, which for painstaking observations and clear interpretation may well rank as a classic of its time. After its original description it remained for nearly three quarters of a century little, if at all known to students of the group, though it is at present a fairly familiar object among its congeners

[1] Gymnophthalmata of Charleston Harbor, 1857.
[2] Bull. Mus. Harvard Coll. Vol. 37 1900 pag. 35.
[3] Memorie Polypi Marini. Napoli 1785.

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of the Neapolitan fauna. It was first studied by the present writer in 1894 and again during the summer of 1903, at which time it was obtained in various stages, from small, single polyps growing among the remains of old and mostly dead colonies of *Eudendrium* and *Pennaria*. This fact naturally recalled the belief of CAVOLINI that it was parasitic upon the former of these hydroids, one of the few points in its original description in which the Neapolitan naturalist was undoubtedly mistaken, as WEISMANN has proven, and as I have also been able to confirm, in that finely developed colonies have been obtained wholly free from any association with *Eudendrium*, or other hydroid, growing indeed upon fragments of rock, etc.

ALLMAN[1] in describing this hydroid, knowledge of which was derived entirely from CAVOLINI's description, expressed somewhat emphatic doubt upon several points of habit, structure, etc. Among them is that of its parasitism referred to in the preceding paragraph. Another had reference to its life history, ALLMAN claiming that it would undoubtedly be found to have a free medusa stage, and further that the claim of CAVOLINI to have traced the origin of eggs and embryos was as undoubtedly erroneous as he believed the other features to have been. He says concerning this point 'It is far more probable that the egglike bodies are parasitical organisms than that they have any direct relation with the hydroid'. He is likewise in error as to the fascicled condition of the stems of the hydroid.

It remained for WEISMANN[2] to show that ALLMAN's contentions were, with the exception of that referring to parasitism, without foundation in fact.

My own observations have confirmed those of WEISMANN on these points without exception.

WEISMANN's account of the essentials of structure, habits, etc., is so full and admirable that it is unnecessary to undertake here to review or repeat it. What follows has to do almost wholly with matters of development, concerning which WEISMANN records little beyond such aspects as come within the province of his particular problem, namely, the origin of the sex-cells.

[1] A Monograph of the Gymnoblastic Hydroids. London 1871 pag. 263.

[2] Die Entstehung der Sexualzellen bei den Hydromedusen. Jena 1883 pag. 35 etc.

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I very much regret that owing to the quality of material and its incompleteness I shall not be able to go into any considerable details as to cytological aspects of maturation or fertilization, though so far as I have been able to observe there seems to be nothing unusual in these respects.

The germ cells arise in the entoderm of the stem and later segregate within follicular pouches where they are nourished and complete their growth, the follicles themselves growing and becoming correspondingly larger, and more or less hydranth-like, but without trace of tentacles, each however having at maturity a protruding head, the end of which becomes ruptured for the extrusion of the eggs. WEISMANN has published admirable illustrations of these features. (Cf. op. cit. Pl. 14, Figs. 1 and 2; Pl. 15, Figs. 3, 4, 5.) The eggs seem to be discharged singly and fertilization probably occurs during this time, following which they become surrounded by a definite firm membrane within which cleavage and the development of the embryo takes place. WEISMANN believes that all the eggs of a given gonophore are discharged at about the same time and that their cleavage and subsequent development proceed simultaneously. In this, however, I am unable to concur. Many cases have come under my observation in which considerable variation in the condition of development of a given cluster of eggs is quite evident. This may be readily seen in preparations of entire gonophores, but is strikingly demonstrated from sections of gonophore clusters. In Figs. 30 and 31 are shown camera sketches of sections of two eggs of the same cluster, in the one case the embryo having reached the period of definite ectodermal differentiation, while the other is only in an early cleavage stage. As compared with other known cases this difference must imply at least a range of from fifteen to twenty hours, and I have no doubt that others of still wider divergence are not lacking. WEISMANN'S citation of the noteworthy approximation to simultaneous development is, however, well made. It is certainly quite unusual to find among hydroids with fixed sporosacs so near an approach to this condition as that under consideration. It resembles more the conditions prevalent in shortlived medusae, like those of *Pennaria*, or *Pachycordyle* as described in a preceding section of this paper. The resemblance is the more striking since there is not the remotest approach toward a medusoid character in these simple follicular sporosacs.

Cleavage begins, as WEISMANN has shown, very soon after the

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discharge of the eggs from the summit of the gonophore. As a rule it seems to be remarkably regular and equal, a condition probably facilitated by the essentially free and independent disposition of the eggs about the conical apex of the gonophore. While during the earlier phases of cleavage the entire egg appears to be involved, in some specimens there appears to be a condition of independent nuclear proliferation similar to that found in many species of Tubularia, among which that cited in a previous section of this paper is an instance. Cleavage finally results in the formation of a solid morula, or in some cases a morula-like embryo having something of a syncytial character, it being impossible to distinguish any definite cellular organization, but rather a multinucleate embryo, such as is well known among not a few Hydromedusae.

Formation of the Germ Layers.

Concerning the ectoderm attention has already been directed to its appearance at a comparatively early period in development. Fig. 31 is one from an embryo which has just begun to assume the piriform shape of the planula. In this it is as yet barely possible to clearly distinguish the columnar shape of typical ectoderm cells of an embryo at this stage. The ectodermal nuclei are definite and large, and for the most part superficially disposed. At a somewhat later period the ectoderm becomes finally and definitely differentiated, and the entire embryo more planula-like, apparently approaching birth. Still I have not found any intrinsic differentiation among the ectoderm cells themselves, such, for instance, as nematocysts.

Concerning entoderm formation I am unable to specify. The entodermal mass shown in Fig. 31 continues essentially unchanged, probably up to and beyond the time of the birth of the planula, a condition not uncommon among Hydromedusae generally. The nuclei continue to proliferate, but without corresponding cytoplasmic activity, at least during the distinctively embryonic development. Differentiation of the entoderm probably takes place by a gradual process of cellular organization of the entodermal mass, parts of which undoubtedly serve, as in other well-known cases, as nutritive substance till the larva finally attaches itself and assumes the polyp stage, while the cellular elements become organized into a definitive entoderm.

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Podocoryne conchicola (Philippi), in part.

Since the original description of *Podocoryne carnea* by SARS[1] in 1846 its distribution has been recorded over a large portion of the northern Atlantic on both eastern and western coasts, and several new species have been described.

During the spring and summer of 1903 the present writer had occasion to examine several colonies of what appeared to be perfectly typical species of *Podocoryne carnea* Sars. An examination of the medusae, large numbers of which were set free in the aquaria, showed them to differ from this species in the rather remarkable fact that they possessed only four marginal tentacles at birth, whereas medusae of the other species have uniformly eight at the time of birth or very shortly following. So far as I am aware, only one species of *Podocoryne* has a medusa whose tentacles are only four in number, namely, *P. aculeata* Rud. Wagner, and in this species all are quite rudimentary and continue so. The specimens here under consideration seem to have four tentacles, and only four. Medusae were kept in the aquaria for several days, indeed as much as a week, during which time the sexual products were matured and discharged, but in not a single case was there the sign of additional tentacles. I was, moreover, assured by Dr. LO BIANCO, who is familiar with the fauna of this region, that this medusa never has more than the four primary tentacles, so far as he had observed.

In his account of British Hydroid Zoophytes HINCKS[2] includes among the species of *Podocoryne*, *areolata*, originally described by ALDER as *Hydractinia areolata*, whose medusae are characterized by the presence of from one to three unequally developed tentacles in each interradial interval, in addition to the four primary perradial tentacles. So far as I am aware this is the only known species whose tentacles number more than eight.

HAECKEL[3] under the generic name *Dysmorphosa* describes four species, namely, *carnea* Sars, *fulgurans* A. Agassiz, *minima* Haeckel, and *octostyla* Haeckel, and these are all characterized by the presence of 8 tentacles, the interradial group usually arising slightly

[1] Fauna Littor. Norvegiae 1846.
[2] British Hydroid Zoophytes. London 1868.
[3] Das System der Medusen. Jena 1879.

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later than the primary, or perradial, and remaining somewhat smaller in size. So characteristic does HAECKEL regard this feature that he has designated it as one of the generic characters of these medusae.

GROBBEN[1], however, has published a somewhat detailed account of *Podocoryne carnea*, one feature of which was the presence of but four tentacles on the medusae. These medusae are represented, moreover, as bearing sexual products, a fact which would certainly imply approximate maturity, and therefore justify the further inference that no additional tentacles appear. GROBBEN's figures have been variously copied into text-books, monographs, etc., but so far as I have been able to discover, no particular note has been made of the apparent anomaly, that all earlier accounts, including, those of SARS, KROHN, HINCKS, ALLMAN, and later of HAECKEL as just cited, give to the medusa the usual eight marginal tentacles. So far as I am aware, nothing has been said as to the probability of this fact being due to the existence of at least a distinct variety, if not indeed of a distinct species.

HAECKEL (op. cit.), has indeed expressed some doubt as to whether there might not be a distinction in the Mediterranean species in the following words: — "Es ist möglich, daß die mediterrane Form von der nordischen echten *D. carnea* specifisch zu unterscheiden ist, in welchem Falle der ersteren der ältere, von ihrem Entdecker gegebene Name: *Dysmorphosa conchicola* verbleiben kann."

WEISMANN has likewise expressed a doubt of a similar sort, as possibly accounting for apparent discrepancies between his version of the origin of the sex-cells and that of DE VARENNE as to the same subject.

From these citations, as well as from the foregoing account, it may be clearly seen that in all probability we have in the Naples form a distinct species, and indeed, on the basis of HAECKEL's System, even a distinct genus. The latter, however, may be at once dismissed as wholly inadmissible, as the mere presence of a few more or less tentacles would not, in itself, justify any such separation. Moreover, when one comes to compare the hydroid colonies of the Naples species with those of other localities there is not the slightest doubt as to their very close relationship. ALLMAN himself says they are indistinguishable from typical *P. carnea* Sars. Incidentally, it may be noted in this connection that ALLMAN's doubt

[1] Sitzungsb. Akad. Wien 72. Bd. 1875.

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as to the presence of the so-called spiral zooids among this species, it not sustained. Both WEISMANN and GROBBEN have shown their presence, and I have found them in every colony examined.

It had occurred to me during the progress of my observations that possibly the tetranematous condition of the medusae of the Naples species might be due to some incidental, or local cause. I therefore examined specimens from various localities, both male and female, but found in every case that the medusae showed the same absolute constancy in this feature. An examination of more than one thousand specimens failed to detect any variation in this respect.

I feel therefore confident that we have here a distinct species, and acting on the suggestion of HAECKEL, previously quoted, I propose for it the name *Podocoryne conchicola*, restoring the specific designation of PHILIPPI[1], who in 1842 described a hydroid under the name *Dysmorphosa conchicola*, which has since been considered by many to have been identical with *Podocoryne carnea* Sars. ALLMAN has, however, doubted the reliability of PHILIPPI's description, and furthermore the widespread use of SARS' name throughout the literature should give pause to any attempt to readjust the matter at present.

The following diagnostic characters are distinctive of the medusa, no occasion arising in my judgment for a redescription of the trophosome, which differs in no significant way from that of typical *P. carnea* Sars (a slightly larger size perhaps, being the only point of difference observed, and that not constant): —

Medusa (Pl. 22 Fig. 26): bell nearly hemispherical, slightly higher than broad, size, 0.8 mm high by 0.7 broad; velum rather wide; manubrium quadrate in shape, with four oral arms which are tipped with clusters of nematocysts; marginal tentacles 4, perradially disposed, and not becoming more numerous with age; when swimming the tentacles usually coiled closely against the bell as shown in Fig. 26, when resting on the bottom of the aquarium or floating sluggishly, tentacles usually extended at right angles to main axis of body; basal bulbs of tentacles rather prominent, and with brownish pigment, though no definite ocelli; outer surface of bell sparingly dotted with nematocysts; gonads borne in four series on the base of the manubrium. Bell very transparent and colorless, except at

[1] Arch. Naturg. 8. Jahrg. 1842 pag. 37.

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base of tentacles as mentioned above; the base of the manubrium is likewise slightly brownish.

As a final note I desire to acknowledge my obligations to the Zoological Station for material used in the foregoing descriptions, and my grateful appreciation of the unfailing courtesy of the Director and other members of the Staff.

Explanation of Figures.

Plate 21.

Fig. 1. Entire colony of hydroid, *Pachycordyle Weismanni*. × 3.
Fig. 2. Medusa of same. Greatly magnified.
Fig. 3. Longitudinal section of stem and gonophore bud, showing egg-cells in entoderm.
Fig. 4. Similar section through gonophore of later stage, showing bell-nucleus *Glk*.
Fig. 5. Similar section of slightly later stage, showing follicular groups of ova, and development of ectoderm of medusa.
Fig. 6. Optical section of medusa about the time of isolation from the coenosarc of stem.
Fig. 7. Section of mature medusa at time of birth. *m*. marginal rim; *v*. velum; *c.c*. circular canal?
Fig. 8. Section through medusa bud some time prior to maturity, showing nutritive entoderm at *ent*, various nuclear features, etc.
Fig. 9. Section of egg of *Tubularia mesembryanthemum*, showing multinucleate condition before cleavage. *n*. nuclei; *ps.c*. pseudocells; *g.w*. wall of gonophore.
Fig. 10. Section of an egg similar to fig. 9, with lines of incipient cleavage at *c.pl*.
Fig. 11. Later stage of multinucleate egg, syncytium.
Figs. 12—17. Outlines of first six sections of an egg, only four of which showed nuclei.

Plate 22.

Figs. 18—23. Similar series, median six sections in a series of fourteen, showing progressive cleavage from animal to vegetal pole.
Fig. 24. Young polyps attached to stem of parent hydroid.
Fig. 25. Colony of hydroid, *Perigonimus napolitanus*. × 10.
Fig. 26. Medusa of *Podocoryne conchicola*, showing position of tentacles during active movements of medusa.
Fig. 27. Colony of *Gemmaria implexa* on shell. × 3.

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Fig. 28. Single hydranth of same, more highly magnified, showing medusa buds at *m*.

Fig. 29. Medusa of *G. implexa*, greatly enlarged, showing general shape of medusa, aspect of tentacles, stalked nematocyst capsules, etc. Only one tentacle drawn complete.

Fig. 30. Section of egg of *Corydendrium* in early phase of cleavage.

Fig. 31. Section of early embryo of *Corydendrium*, with ectoderm, *ect.* Undifferentiated entodermal mass *ent.* comprising the center.

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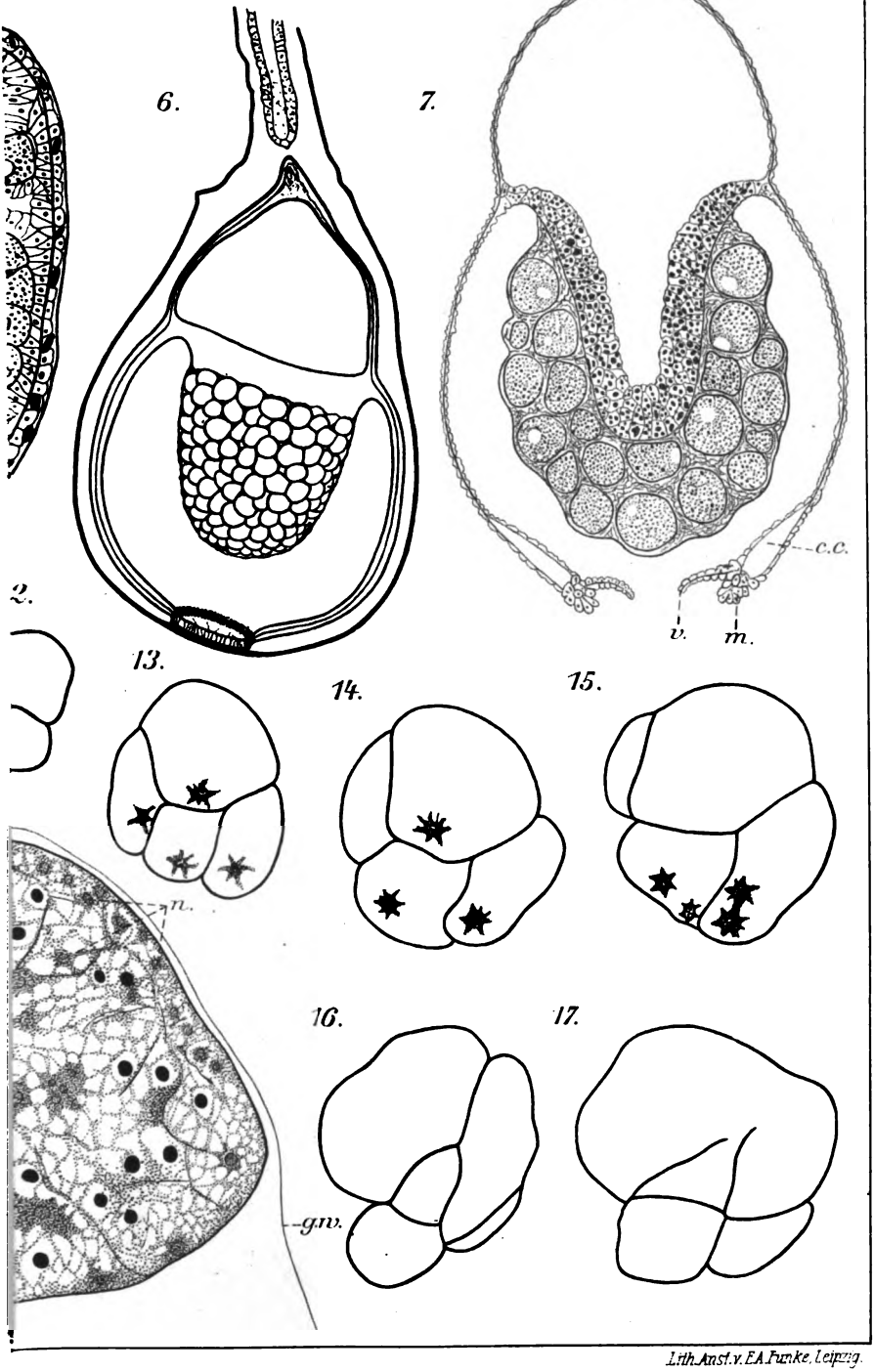

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Some Observations on the Chromosome Vesicles in the Maturation of Nudibranchs[1].

By

W. M. Smallwood.

With Plate II.

These observations were made on three species of Nudibranchs collected at Wood's Holl during the summer of 1902. While several different forms were secured, the eggs were not obtained for all of them in sufficient quantity to permit of a detailed study of the problem of maturation in each. This paper then, is confined to certain phases of maturation in the eggs of *Doris bifida*, *Montagua Gouldii*, and *Montagua pilata*.

No attempt will be made to give a full account of maturation but only of those changes that especially accompany the history of the chromosomes; nor will the general literature[2] be reviewed except in so far as it bears on the special problem of this paper.

The spawn of *Doris bifida* is long and ribbon like, 3/8 of an inch wide; one ribbon was two inches in length. The egg mass was always found attached to some foreign object, such as the common Fucus or eel-grass. The form of the spawn was interesting; in several instances the lower margin was attached to the stem of the Fucus and while laying the animal had turned nearly around so that the band of eggs had the appearance of an open flaring collar. This was a common shape in the Fucus, but occasionally the spawn did not have any definite form, being attached to several

[1] Contributions from the Zoological Laboratory, Syracuse University.

[2] For full review of the literature of maturation see CONKLIN, 02, and SMALLWOOD, 04.

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branches. The eggs are arranged in regular rows at right angles to the long axis of the spawn. KORSCHELT and HEIDER[1] estimate that 600 000 eggs are laid by each animal. Deposition occurs from twelve to twenty four hours after copulation. At the time when the eggs are laid, they are in the metaphase of the first maturation spindle. The succeeding changes follow rapidly and within an hour or an hour and a half the eggs have segmented into the two and four celled stages. This is a great help in the study of this problem for a single spawn mass may show, if fixed in the metaphase of the first maturation spindle, all of the changes involved in the formation of the first and second polar bodies.

In the breeding season *Montagua Gouldii* are found in the hydrorhizal portion of *Tubularia crocea*, being especially abundant in deteriorating colonies; usually *Panopeus*, *Dendronotus*, young *Mytilus*, and many larvae of Worms, Crustacea, etc., are found in company with them. The spawn is small and fan-shaped, each normal sized mass containing about 500 eggs. After a few hours the mass assumes an oval form but if it is kept in stale water, the eggs die, the egg mass agains assuming the fan-shape with the eggs arranged in radiate rows. The spawn is either scattered in Tubularia or laid in nests; that is, in small spaces free from the stems and branches of the hydroid. In these nests from 50 to 100 masses of spawn are found. Each individual lays a variable number of egg-masses. A record of 157 animals (SMALLWOOD, '03) showed an everage of 6.2 spawn-masses for each animal after being brought into the aquarium. Deposition occurs about the same time after copulation as in *D. bifida*. The rate of development is slow in comparision with the previous species. While the maturation phenomena are well started when the eggs are laid, yet the second polar body is not formed until four hours have elapsed after deposition; all of the eggs in a single spawn mass are in about the same stage of development. A rest period of about two hours exists between the two and four celled segmentation stages.

Montagua pilata were taken by dredging in Vinyard sound. The method of copulation is somewhat different from the two mentioned above. Ample opportunity was afforded to observe the process which is as follows[2]: »The ends of the oviducts become firmly

[1] Invertebrate Embryology. Part IV. p. 102.

[2] SMALLWOOD, 03, pp. 15, 16.

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united, probably with mucous, so that the animals may be pulled about in the aquarium with considerable freedom without causing them to separate. I took a pencil and pushed one animal of a pair all around the dish but they did not separate until the irritation became extreme. When they are copulating the distended and swollen ends of the oviducts as they are united are spherical in form and the color is intensified. So close is the union that I could not detect the line of fusion. The distended end of the oviducts after copulation is gradually withdrawn into the body until the animal presents a normal appearance; this reduction took nearly three hours in one instance.«

»The egg-mass of *M. pilata* is laid in the form of a string which in some instances was at least six inches long. This string of eggs is more often undulating than straight. There are from two to eight eggs in a single section of the string. No attempt was made to determine the whole number of eggs laid by one animal. The animals in confinement frequently laid short strings of eggs, one to three inches in length and laid several times. Deposition occured from twelve to twenty-four hours after copulation. The rate of development of the eggs is the same as *D. bifida.*

Methods. No difficulty was experienced in securing properly fixed material by the use of KLEINENBERG's picro-sulphuric fluid, BOVERI's picro-acetic, and GILSON's mixture; although the latter gave the least satisfactory results. Many of the spawn masses of *M. Gouldii* were very difficult to section when fixed by either of the above reagents. The following stains were used: HEIDENHAIN's Iron haematoxylin accompanied by a plasma stain of Bordeaux red, CONKLIN's picro-haematoxylin, and Brazilin. Each have their especial value, the first two being used on all of the stages described in this paper. So tenaciously does not only the deutoplasm but also the cytoplasm retain the Iron-haematoxylin stain, that it was impossible to secure a differential stain by its use alone. The eggs of the two species of *Montagua* are very difficult to stain satisfactorily. The eggs were imbedded in paraffine at 53 degrees F. but left in the warm paraffine only thirty minutes which was found to be adequate for penetration and did not cause the eggs to become friable.

Ovocyte. Sections of the ovotestis of each of the three species shows the ova in all stages of development. The young ovocyte is free from deutoplasm, the cytoplasm presenting a granular appearance, homogenous throughout. The increase in size is accompanied by the

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presence of deutoplasmic bodies in the cytoplasm. The growth is gradual and the material taken up is apparently in a liquid state as it enters the ovocyte. The deutoplasm increases in amount until it completely fills the region between the nuclear wall and the cell membrane, thus concealing the structure of the cytoplasm. Before the egg is laid, the germinative vesicle is central in position.

The changes in the nucleus may be readily observed. The nucleolus in a young ovocyte is a solid mass which takes a deep stain even before the nucleoli in the cells of the other parts of the body show much reaction to the same stain. A number of prominent linin fibres bear chromatic material. As the deutoplasm appears in the cytoplasm, small clear regions may be distinguished in the nucleolus, if the stain is not too intense. This is the beginning of the vacuolization of the nucleolus, the further metamorphosis of which is similar in effect to the changes in *Piscicola repax* and *Tetrastemma elegans* (MONTGOMERY, 99), and *Haminea solitaria* (SMALLWOOD, 04); but the process is different in so far as it concerns the method by which the chromatin is displaced. After the vacuoles begin to appear, the solid mass of chromatin breaks up into many small bodies (Fig. 1). This fragmentation of the nucleolus is accompanied by a decrease in the amount of chromatin until it disappears entirely. The loss of chromatic material from the nucleolus prevents its fate from being followed during the early stages of maturation; when the chromosomes are forming from the germinative vesicles, no trace of it is evident.

Maturation. The earliest indication of the first maturation figure was found in a spawn-mass of *M. Gouldii* that was fixed together with the animal as the spawn was being deposited. The eggs for a few minutes after they have been laid are irregular in shape, none in *M. Gouldii* being spherical. The eggs appear as if they had been forced through an opening too small for them and as a result had become elongated and kidneyshaped. Dentalium exibits a similar irregularity in shape but apparently not as marked (WILSON, 04). While in this irregular form, the germinative vesicle is found in any region of the ovum. Maturation begins at this time in this species. In Fig. 2, a large portion of the germinative vesicle is present, the spindle fibres having caused the wall on one side to disappear. The chromosomes are forming as independent bodies; not all are in the same stage of development, that is, some are smaller and take a lighter stain. The germinative vesicle has in-

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creased in size and reveals a sharp differentiation into two substances, basichromatin and oxychromatin (see CONKLIN, 02, p. 9), the former becomes the chromosomes, the latter spindle rays, sphere substance and cytoplasm.

No evidence of a chromosome vesicle was found in these early stages; each chromosome forms from a single small particle that increases in volume as maturation continues. In fig. 3, these bodies are more distinct and associated with the spindle fibres. The network of linin and oxychromatin substance is less and there is a corresponding increase in the number of the spindle rays. The next stage represented (Fig. 4), shows the chromosomes approximately full sized and in the region of the equator of the spindle.

In all three species the chromosomes in the metaphase assume a variety of shapes (Fig. 5). This irregular condition gives way to a simple rod-like chromosome which appears to divide transversely in this and the second maturation spindle. CONKLIN (02) believes that the irregularity may be caused by the fact that the chromosome vesicles confine the chromatin to certain regions. He finds in Crepidula chromosome vesicles (Plate I, Fig. 12 *a*) at this stage. I have not been able to detect in these three species of Nudibranchs similar structures. In discussing the irregularities in the form of the chromosomes he says (pp. 11—12): »It is difficult to say whether these differences in the shapes of chromosomes mean much or not. On the one hand it is possible that all the chromosomes of a given mitosis cannot be reduced to a single type; that their differences in shape indicate differences in material substance, and that different chromosomes may therefore represent different heritable qualities. On the other hand these differences in the shapes of the chromosomes are generally limited to the first maturation division; they are rarely found in the second maturation and only to a limited extent in cleavage. Furthermore, there are many evidences that the shapes of chromosomes are conditioned by their linin sheaths, and that the chromatic substance which fills the sheath is of a semi-fluid or viscid character.« My observations on the early maturation stages in the Haminea may throw some light on the suggestion quoted. In this mollusc the chromosomes assume an irregular form while in the chromosome vesicles; and further, they are irregular on the free surface and much smoother on the side or parts in contact with the linin sheath. In this species the chromosomes do not fill the linin sheath in the prophase stage which affords an apportunity to note

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the change in the form of the chromosome. Here the irregularity seems to be an inherent quality of the chromatic material itself, independent of the chromosome vesicles (linin sheaths).

I have counted the number of chromosomes in several eggs in each species and believe the number to be 16 during maturation and 32 in the segmentation stages.

Before the second maturation figure is completed, the chromosomes usually pass through interesting changes. During the late anaphase of the first maturation, the chromosomes lie crescent-like in the region of the deep centrosome (Figs. 6, 7, 8), but show no evidence so far as was observed of passing into a vesiculate state. At about the time that the young amphiaster of the second maturation figure is forming and moving into a radial position, a distinct membrane appears around each chromosome in many eggs (Figs. 9, 10). These chromosome vesicles lie so near the chromosomes as to be overlooked in many instances. It frequently happens that one chromosome vesicle contains two or more chromosomes (Fig. 9) in which case the chromosomes are united by narrow strands of chromatin.

That the chromosomes do not always pass into chromosome vesicles and undergo these important changes is shown by the conditions in Fig. 11. Here the chromosomes lie free in the cytoplasm and it is believed by the writer that this is a perfectly normal condition and that there may be two distinct processes in the maturation of these Nudibranchs just as there are in *Haminea* (SMALLWOOD, 04). The process free from the containing vesicles is about the same as that which occurs in most animals. The conclusion that there are these two rather distinct processes in maturation is not reached without studying a large number of eggs in both states and when it was found impossible to harmonize all of the conditions I was forced to arrange the phenomena under two heads. In describing similar changes in the egg of *Haminea* the following distinction between the two processes was made. »The two processes occur with about the same frequency, but all the eggs in one capsule follow either one method or the other. Any division of these processes into successive stages must be more or less arbitrary, especially since there are no periods of rest, the changes being both continuous and rapid. Until the centriole at the deep end of the first maturation spindle has divided and the chromosomes of that spindle have begun to move apart there is nothing to indicate that there are two processes, except possibly a difference in the size of

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the centrosomes. According to the degree of complexity of the changes which accompany this stage and the length of time required for the metamorphosis, the processes may be designated as the shorter or direct and the longer or indirect. The shorter process is characterized by the chromosomes remaining free in the cytoplasm and by the direct formation of the achromatic figure; while in the longer process the chromosomes become enclosed in a so-called quiescent nucleus and the achromatic figure is formed indirectly.«

As a rule the chromosomes in the first polar cell do not possess vesicles until after the division of this cell but an exception is represented in Fig. 10, where two of the chromosomes are enclosed but there is nothing to indicate that the remaining ones undergo a similar fate.

Before the second maturation figure is fully formed, the chromosomes show important changes in the longer or indirect process. (The shorter or direct process is not described in this paper because it is so similar to the same phenomena in other Mullusca and many Annelids.) Each of the small chromosome vesicles with its contained chromosome looks like a minature nucleus. The chromosome is less prominent than in the previous stage (Fig. 10) and there exists definite linin threads between it and the containing wall. The early stages in these changes are indicated in Fig. 12. It may be noticed in passing that the marked spindle between the two centrosomes has disappeared, a phenomenon that will be fully considered later.

The conditions represented in Fig. 13, show the body that was the chromosome in Fig. 10, changed into one or possibly several small granules of chromatin lying in the meshes of the linin. In the transition from this unusual state to a solid, large chromosome, the changes are the same as those which occur when the chromosomes form from the germinative vesicle. The chromatin granules increase in their staining capacity growing in the mean time until they are full sized again. As to the fate of the limiting vesicles two possibilities may be offered (s. CONKLIN, p. 12), either they persist as indistinguishable sheaths or dissolve and loose their identity in the cytoplasm. These fully re-formed chromosomes lie in the equatorial plane of the spindle, divide, pass to the poles of the amphiaster, the egg chromosomes again passing, or forming, as is usual, into vesicles which sooner or later fuse to form the egg nucleus.

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The chromosomes which are in the polar cells pass through a number of states which so far as I am aware have not been previously recorded. They are as follows: When the first polar cell is formed the chromosomes, as already stated, do not usually pass into a vesicular condition until after its complete division. These two polar bodies loosely attached to the cell wall frequently are pushed to one side during the formation of the second polar cell. In Fig. 15, the chromosomes are still free in the polar cell, while in Fig. 18, they lie enclosed in a definite nuclear vesicle and have lost some of their distinctive appearance and are associated with linin fibres. The changes connected with the chromosomes in the polar cells can not be correlated in time with similar changes in the egg chromosomes.

Clearer evidence in regard to these changes was furnished in the second polar cell. This cell is often quite large (Fig. 22), so that its condition can be readily observed. Each chromosome is surrounded by a vesicle so that we may have as many chromosome vesicles as there are chromosomes, or all or several pass into one vesicle. In Fig. 15, the egg chromosomes are in separate vesicles having linin fibres present. There is not the close grouping usual at this stage. This is further shown in Fig. 16, where considerable cytoplasm intervenes between some of the vesicles. But in the second polar cell (Fig. 15) the chromosomes all lie in one vesicle, showing no further evidence of entering a nuclear state, the presence of linin fibres and the ordinary differential conditions present being entirely absent here. While the conditions in Figs. 16 and 18, reveal but a single vesicle for the second polar cell yet there is plenty of evidence of linin fibres and the chromatin gives a differential reaction to haematoxylin in that certain of the granules take a very heavy stain while others react but slightly.

The character and number of the chromosome vesicles formed seems to vary with each individual ovum. A few typical conditions are represented in Figs. 17, 19, 20 and 22. These vesicles may be coördinate in size and position or very unequal (Figs. 17, 20).

In Fig. 17, there is a large and prominent nuclear vesicle in the polar cell in a typical nuclear state; while in the narrow part, toward the egg, there is a single small vesicle containing a chromosome. No evidence of linin fibres could be distinguished, there was, however, a slight stain noticable in the contents of the vesicle between the wall and enclosed chromosome. I believe this to be

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a chromosome that was not included with the rest in the large vesicle. Two chromosomes are frequently included in a single vesicle (Figs. 19, 22), in which case the changes are the same as when one chromosome occupies a single vesicle. In Fig. 20, we have all the chromosomes in three vesicles which are so large that they almost completely occupy the polar cell. In this instance several of the chromosomes still retain their individuality but we can not follow the fate of a single chromosome in these vesicles any more than we can in the egg nucleus in Figs. 19 or 20.

Figs. 21 and 22 are from the same ovum being adjacent sections. There are in the polar cell in Fig. 22 three bodies which take a plasma stain and look much like chromosome vesicles. I think that these three bodies are not vesicles but are to be interpreted as partly metamorphosed deutoplasmic spheres that have passed into this polar cell. Evidence for this conclusion is furnished by the character of a similar, although larger body in the egg itself, of this same figure, lying near the male nucleus which is undoubtedly a yolk sphere. Furthermore, it is not unusual for yolk bodies to be found in the polar cells in Mollusca, and especially in these three Nudibranchs. I have further noticed in segmentation stages that the deutoplasm did not take as heavy a stain as it became partly metamorphosed into cytoplasm.

Discussion. Chromosome vesicles similar in many particulars to the one here described are also found in the spermatogenesis of *Brachystola magna* (SUTTON, '01) They occur in late anaphase and the contained chromosome retains its identity more definitely than in the cases cited in this paper. LILLIE ('02), finds in experimental work on *Chaetopterus* eggs that chromosome vesicles may appear around each particle of chromatic substance. In discussing this phase of the question he states (p. 490) »Even the smallest discernible particles of chromatin exercise, apparently, a liquifying effect on the cytoplasm, and thus appear to lie in vacuoles (Fig. 27), a condition analogous to the return of the nucleus to its resting condition«. CONKLIN describes for *Crepidula* chromosome vesicles (linin sheaths) in the metaphase of the first maturation but does not find them at other stages. HARGITT ('04) finds in the natural development of *Pennaria tiarella* chromosome vesicles similar to the ones in the eggs of *Chaetopterus* and these Nudibranchs. Chromosome vesicles are found then in both normal and artificially developing eggs. That such unusual changes should occur in both normal and artificial

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development is interesting and raises the question of the significance of these structures and also the further question: Are we to regard such phenomena as found in the eggs of *Chaetopterus* as entirely the result of the artificial treatment?

In *Haminea solitaria* chromosome vesicles are present in the early prophase of the first maturation. In this mollusc it does not matter whether the eggs have passed into the oviduct and have been fertilized, or are still in the follicles of the hermaphroditic gland, maturation begins in all as soon as the first is deposited. The penetration of the sperm is not the force which starts the mitotic phenomena but apparently it is the contraction of the body in forcing the eggs to the outside which incites the formation of the maturation figure. It frequently happens that one may find in the ovotestis of *H. solitaria*, if fixed when deposition is in progress, eggs in the metaphase of the first maturation figure in the most distant follicles. Several hundred eggs all in the same stage are found, indicating that these changes started in each at the same time. A study of these eggs which have just begun maturation reveal chromosome vesicles which are to contain or do already enclose a single chromosome.

In the earliest stages these vesicles lie at the ends of the spindle fibres. Irregular in shape, they may contain several or but one chromatin mass. The number of vesicles which arise corresponds to the number of chromosomes. As they enlarge each becomes elliptical in outline, the chromatin increasing in quantity at the same time. A more extended study of these stages seems to indicate that their origin is in the linin. When these vesicles first appear, they are in their largest state although not as definite or as thick walled as in the metaphase (Fig. 12, Plate 3). The chromatin in the early prophase is in the form of small granules located at the nodes of the linin meshwork. While it is possible that the formation of the vesicles may involve a process of vaculolation around the chromatin yet no difference in the chromatin located at the nodes of the linin fibre and that enclosed in the crudely formed vesicles can be noted. Whenever the chromatin is present along the walls of these vesicles, it can be readily distinguished by any of the basic stains. As the linin disappears from the germinative vesicle in the early prophase, the number of vesicles increases; probably, however, a portion of the disappearing linin, contributes to the formation of spindle fibres and sphere substance as in *Crepidula*. The evidence in *H. solitaria*

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seems to indicate that the vesicles which I have termed »chromosome vesicles« are formed from the linin, the details of which process I have as yet not been fully able to solve.

But whether the vesicles which occur in *H. solitaria* are the lame as those in *Crepidula* and in *Doris* I have some doubt. That the vesicles found in *Crepidula* and *Doris* are the same there can be no question. The following may be said concerning the fate of these vesicles in *Haminea.* The chromatin increases in quantity for a time but never completely fills the vesicle, a clear space which takes a plasma stain is always evident. At the time when the chromosomes have become full sized or as large as they do become while in the vesicles, there is no evidence of a shrinking of the walls or a diminution of the enclosed area; the only change being that the walls become less distinct.

While this discussion must be tentative at this time as the facts are too few to warrant any definite conclusion, yet it is difficult for me to see how these vesicles can persist through the subsequent maturation changes as an indistinguishable limiting membrane around each chromosome as suggested by CONKLIN. But is seems to me rather that the walls of the chromosome vesicles disappear, simply fading away, probably being dissolved in situ. This leaves us with two suggestions. 1. That the linin sheath lies close to the chromosome and is evident only during division. CONKLIN, p. 12. 2. That the chromosome vesicles in *H. solitaria* disappear in the cytoplasm being dissolved in situ. The origin of these vesicles in *Doris* will be further discussed in connection with the problem of fertilization.

Centrosome. In describing this body the terms proposed by BOVERI ('01, p. 32, 125) are used. The centrosome is the body in which the astral and spindle fibres terminate. At certain stages it is a highly differentiated body composed of a central granule, termed the centriole which is surrounded by an area designated the centroplasm. The centroplasm usually takes a plasma stain while the centriole is always stained by haematoxylin.

In the eggs of these three species of Nudibranchs no evidence of a centrosome was found before ovoposition had begun; nor was it possible to distinguish with certainty this body in a stage earlier than that shown in Fig. 2. In Figs. 2 and 3, the centrosome is a small, solid granule, undifferentiated. None of the stages indicate that this body is composed originally of more than one body as in

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Unio (LILLIE, '01), *Crepidula* (CONKLIN, '02), or *Haminea* (SMALLWOOD, '04). There is surrounding the centrosome a dense sphere substance which renders it difficult to be certain how far the astral rays extend but in some of the faintly stained eggs, the rays can be traced to this central body. The centrosome becomes early differentiated into centroplasm and centriole. Within the sphere substance and close to the centrosome, a clear area begins to appear through which the rays and fibres do not pass. This is the first indication of the centroplasm; it is apparently formed from the solid centrosome and increases in size until it is several times as large as the early stage shown in Fig. 3. The centroplasm fully formed takes a very heavy plasma stain (Figs. 5, 6). When stained with iron haematoxylin and before the subsequent differentiation with the iron, the deep (egg) centrosome frequently looks like a large, irregular precipitate of haematoxylin which may be so tenaciously retained that in removing it all of the other structures are completely de-stained. This precipitate occurs but rarely in connection with the outer centrosome. Before discussing the further structural changes that occur in the centrosome, we will consider briefly the astral rays, spindle fibres, and sphere substance.

A few astral rays are present around the centrosome in Fig. 2; these increase rapidly in number and prominence as the germinative vesicle disappears, until in Figs. 4 and 5, the typical astral appearance is produced. These rays seem to be of the same character and nearly of uniform length, there bing no sharp differentiation into primary and secondary rays in *Haminea*.

The growth of the spindle fibres is interestingly shown in Figs. 2 and 3. The rays which are to unite the two centrosomes and become the central spindle proceed directly to this union (Fig. 4). The mantle fibres few in number in Fig. 2, are the most conspicuous of any of the rays at this stage. They extend into the meshwork of linin fibres where the isolated chromosomes are forming. More of these fibres are present in Fig. 3; while in Fig. 4, they have become very numerous and the linin meshwork has entirely disappeared, the two events being intimately associated. The completely formed spindle having the chromosomes in the equatorial region is shown in Fig. 5.

An undifferentiated sphere substance appears in each of these Nudibranchs especially in the prophase of maturation but it does not become clearly defined into two layers as in *Haminea* and

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others. In the metaphase and anaphase no definite sphere substance is noticed, it is again evident in the early prophase of the second maturation (Figs. 8, 9, 10), a slight trace of it is found in Fig. 14, but there is no marked evidence of its presence in Figs. 12 and 13. In a few instances in the telophase of the second maturation, the nuclear vesicles are surrounded by a clear area (Figs. 17, 19, 21), this I take to be the sphere substance of CONKLIN and others.

The first evidence in the centrosome of the prophase stage of the second maturation is the division of the centriole into two equivalent parts. At first they lie close together (Fig. 7), but soon separate, remaining connected by more or less regular strands which take a basic stain (Fig. 8). These two centrioles with the potential spindle uniting them give rise in a large measure to the second maturation figure. With the increase in size of the centrosome, the centroplasm creases to take a plasma stain, soon breaks appear in the wall and astral rays form about the centrioles. The different positions in the centroplasm which the forming spindle assumes demonstrate beyond a doubt that in these Nudibranchs the old wall does not contribute to the formation of the new centrosomes as is held by MACFARLAND ('97) but the formation of the spindle does agree with similar changes in *Crepidula* and *Haminea*. While there is no particular rest period or division into distinct stages in this metamorphosis of the centrosome, for convenience in description, we may now apply the term centrosome to the bodies previously designated as centrioles although still surrounded by the centroplasm of the old centrosome. The fibres that unite the two new centrosomes become more prominent and longer. The wall of the old centrosome breaks up into smaller and smaller pieces until it becomes indistinguishable from the granules in the cytoplasm; the centroplasm likewise disappears. The old astral fibres and rays do not persist as such and become a part of the second maturation figure. The new spindle has more prominent fibres on the periphery than within, the central ones frequently branching an anastomosing.

In Fig. 9, the two centrosomes are each a single body; while in a later stage, each is composed of centriole and centroplasm, these parts being derived as already described in the first maturation. The further history of the centrosome in the second maturation figure is the same as in other mollusca, disappearing entirely as the chromosomes become transformed into the female pronucleus.

The further changes in the spindle are parallelled so far as I

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know only by a similar metamorphosis in *Haminea*. As the maturation figure becomes radial in position and of a length approximating the second figure, the spindle formed from the two centrioles breaks away and is lost in the cytoplasm. The two asters now separate, the deeper one moving nearly to the centre of the egg. A new spindle must be formed from the cytoplasm. The first appearance of these new fibres is a linear arrangement of granules which become fused into a single fibre (Fig. 12). These continue to form in a similar manner until a new spindle appears (Figs. 13 and 14). Although the old astral rays remain, new ones are formed in the same way that the spindle fibres arise. The full details of this unusual process are presented in my paper on *Haminea* (Figs. 46—51). The conditions are the same in *Doris* and *Haminea* in all of the changes except one. This exception in *Haminea* consists in the formation of unmistakable fibres proceeding from the centrosomes while they are still enclosed in a vesicle; these are rays which are in addition to the ones formed from the cytoplasm. No such process was discovered in the growth of this new spindle in *Doris*.

Fertilization. Although it was not the purpose of this paper to make a detailed study of fertilization yet the following facts were noted while observing the maturation phenomena. The sperm head penetrates the ovum in the oviduct just before deposition. The tail of the sperm is left outside. As soon as the head has entered the egg, it increases in size and is usually slightly bent near the middle. A few small granules close to the sperm head stain more intensely than the rest of the cytoplasmic granules but I was not able to determine their origin. The sperm head moves across the egg toward the animal pole, its path depending on the place where it entered the egg. In this approach to the animal pole the sperm nucleus becomes vesicular in the usual manner. But while passing through these changes, the chromatin may be confined in one vesicle (Fig. 18) or several (Figs. 15, 17, 21). In most cases there is one quite large vesicle and a few small ones. The large one eventually absorbing the others. Structurally there is no essential difference between these different vesicles aside from their size. In Fig. 17. the two small sperm vesicles, each containing a bit of chromatin, have the same appearance as those represented in Figs. 9—10, These sperm vesicles seem to be derived from the cytoplasm forming under the influence of the chromatin. When the chromatin remains

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compact, one vesicle forms but when it becomes scattered, several may appear.

The sperm nucleus in Fig. 18, presents one structure, the meaning of which is not clear. In this section of the nucleus there are three sharply defined masses of chromatin, one of which is free from any connection with linin fibres. The other two are surrounded by a substance that takes a distinct plasma stain and the edge of which is continuous with the linin fibres. It looks as if there were here an extra amount of substance similar to linin and from which linin arose concentrated about these two chromatic masses. To summarise we may say that vesicles similar in appearance and content are found during the prophase stage of the second maturation; in the polar cells; and in connection with the male and female pronuclei.

In addition to the usual maturation phenomena in these Nudibranchs, a portion of the cell contents may be budded off without the mitotic or amitotic process. Just after the eggs are deposited or even while the animal is laying, the eggs may, while changing from an irregular to a spherical form, give off a large part of the cell, a fourth or fifth of the whole cell is thus given off. The formation of these small cell like bodies was observed in many spawn masses of these Nudibranchs. They appear before the first polar cell has had time to be formed. While observations were being made on the living eggs, these large bodies were thought to be simply unusually large polar cells.

In a study of the sections of *M. Gouldii* I was fortunate in securing two spawn-masses in which a number of the eggs were undergoing the formation of these peculiar bodies. Fig. 23 is a drawing made from two adjacent sections showing this cell-like body still attached to the egg and the first maturation figure in the prophase stage. The position of each is such that there can be no possible connection between them. The budded cell-like body is full of deutoplasmic spheres, and a few cytoplasmic granules are evident. But there is no evidence of a nucleus or of free chromosomes either in this body or the cell proper apart from the maturation figure. An examination of all of the sections of this egg with a Zeiss homog. aprochromatic 2 mm lens and compensating oculars as well as with the 1/12 oil immersion lens failed to reveal any structure which might be designated as a nucleus or chromosome or chromatic substance in connection with the formation of this cell-like

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body. A number of other eggs showing the same cell-like bodies confirmed these observations.

This cell-like body does not remain attached to the cell proper for any considerable time. While studying it in the living condition, no evidence of segmentations was ever found. These bodies, it seems to me, can have but one fate, i. e., to disintegrate probably furnishing food for the veliger.

In *Haminea solitaria* similar small cells occasionally appeared just before the first cleavage the fate of which was not determined.

During the early cleavage in many Gastropods, Lamellibranchs and Annelids there is formed at the vegetal pole a lobe of varying size. This lobe is simply a bulging out of a portion of the egg contents and was first designated as a yolk lobe (polar lobe WILSON, p. 12). The conditions represented in Fig. 23 differs in two marked respects from this so-called yolk lobe; first, the time when it appears, and secondly, its position. The fact that these bodies are seen to form while the egg is becoming spherical in outline and long before cleavage begins indicates that this phenomenon is distinct from the yolk lobe. So far as I am aware, the yolk lobe is always formed in the region of the vegetal pole while these bodies do not occur at any one definite place on the egg. Some were formed near or at the vegetal pole, others occured equally near the animal pole.

As soon as opportunity is afforded, the writer hopes to extend these observations.

Summary.

1) The eggs of *Doris bifida*, *Montagua Gouldii* und *Montagua pilata* may be obtained during the months of June and July at Wood's Holl, Mass. Each of these three species will lay under ordinary aquarium conditions in from 12—24 hours after copulation. The eggs are deposited in a gelatinous mass characteristic in form for each species. The number of eggs and egg masses that each animal lays is variable.

2) The growth and changes in the ovocyte are, in the main, those characteristic of *Mollusca*.

3) The beginning of maturation is co-incident with deposition.

4) The chromatic substance is differentiated into basichromatin and oxychromatin. The basichromatin gives rise to the chromosomes which make their appearance as independent bodies; the oxychro-

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matin passes into the cytoplasm to contribute in part to the formation of the sphere substance.

5) No evidence of chromosome vesicles was found during the prophase of the first maturation as is the case in *Haminea solitaria*.

6) During the »rest pause« between the first and second maturation, the chromosomes frequently have distinct vesicles. There may be a single vesicle for all of the chromosomes or a single vesicle for each chromosome. All combinations between these two extremes occur.

7) The chromosomes that pass into the polar cells likewise possess vesicles which in some instances exhibit all of the structural features of a typical nucleus.

8) The egg chromosomes enclosed in vesicles change while in this state until each vesicle has the appearance of a miniature nucleus. Before the metaphase of the second maturation, the several chromatic granules unite into a solid mass, the vesicle probably disappearing.

9) The fact that the chromatin may normally exist in a liquid state during the prophase of the first maturation of *Haminea solitaria* and in the prophase of the second maturation of these Nudibranchs strengthens the growing conviction that the theory of the qualitative division of the chromosomes is untenable.

10) The growth and fate of the several parts of the centrosome are in close agreement with these same changes in the centrosome of *Haminea solitaria*.

11) Fertilization takes place in the oviduct, the tail remaining outside. The sperm head becomes vesicular during its progress towards the animal pole and while it is undergoing this change one or more chromosome vesicles may be formed in connection, with the chromatin derived from the sperm, which are similar to the egg chromosome vesicles. These vesicles arise undoubtedly through the influence of the chromatin on the cytoplasm which fact suggests that the vesicles in these three Nudibranchs and those in *Haminea solitaria* which arise in the nucleus are not identical.

Zoological Laboratory, Syracuse University,
May 1st, 1904.

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Explanation of Plate II.

All figures are camera drawings and were drawn at the base level under the ZEISS 1/12 oil immersion, Oculars 2 and 4.

Abbreviations.

chr Chromatin,
chr'so Chromosome,
c'l Centriole,
cl.pol.1 first polar cell,
cl.pol.2 second polar cell,
c'pl Centroplasm,
deu'pl Deutoplasm,
fus.c Central spindle,
nll Nucleolus,
pronl.♀ Female pronucleus,
pronl.♂ Male pronucleus,
vsl.chr'so Chromosome vesicles.

Fig. 1. Ovocyte from ovotestis. Deutoplasmic spheres quite abundant in the cytoplasm. Nucleolus vacuolated and the remaining chromatin granular. 1/12,2.

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Figs. 2, 3. Early prophase of first maturation. A large part of the germinative vesicle still intact. Chromosomes forming as isolated bodies in the meshwork of linin. 1/12,4.

Fig. 4. Prophase first maturation. Germinative vesicle completely absorbed. Chromosomes rod-shaped and forming in the equatorial region of the spindle. 1/12,4.

Fig. 5. Metaphase of first maturation spindle. Chromosomes rod-shaped and of irregular outline. Centrosome differentiated into centriole and centroplasm. 1/12,2.

Fig. 6. Anaphase first maturation spindle. 1/12,2.

Fig. 7. Anaphase first maturation spindle showing the division of the centrioles. 1/12,4.

Fig. 8. Anaphase first maturation spindle. Centrioles in deep centrosome are united by fibres. Prophase stage in the division of the first polar cell. 1/12,4.

Figs. 9—10. Prophase second maturation spindle. Chromosomes enclosed in chromosome vesicles. The indirect or longer process in the formation of the second polar cell. 1/12,2.

Fig. 11. Prophase second maturation spindle, shorter or direct process. 1/12,2.

Figs. 12—13. Prophase stages in the formation of the second maturation spindle by the indirect process. Chromosomes in the form of chromatin granules enclosed in chromosome vesicles. 1/12,2.

Fig. 14. Metaphase in second maturation spindle. Polar cell in same stage. 1/12,4.

Figs. 15—22. Telophase second maturation. The egg-chromosomes lie either in a single vesicle or in several. The chromosomes of the polar cell are found in a single vesicle or several. Figs. 15—20, 1/12,2; Figs. 21, 22, 1/12,4.

Fig. 23. The prophase stage of the first maturation showing a large cell-like body in the process of formation. 1/12,2.

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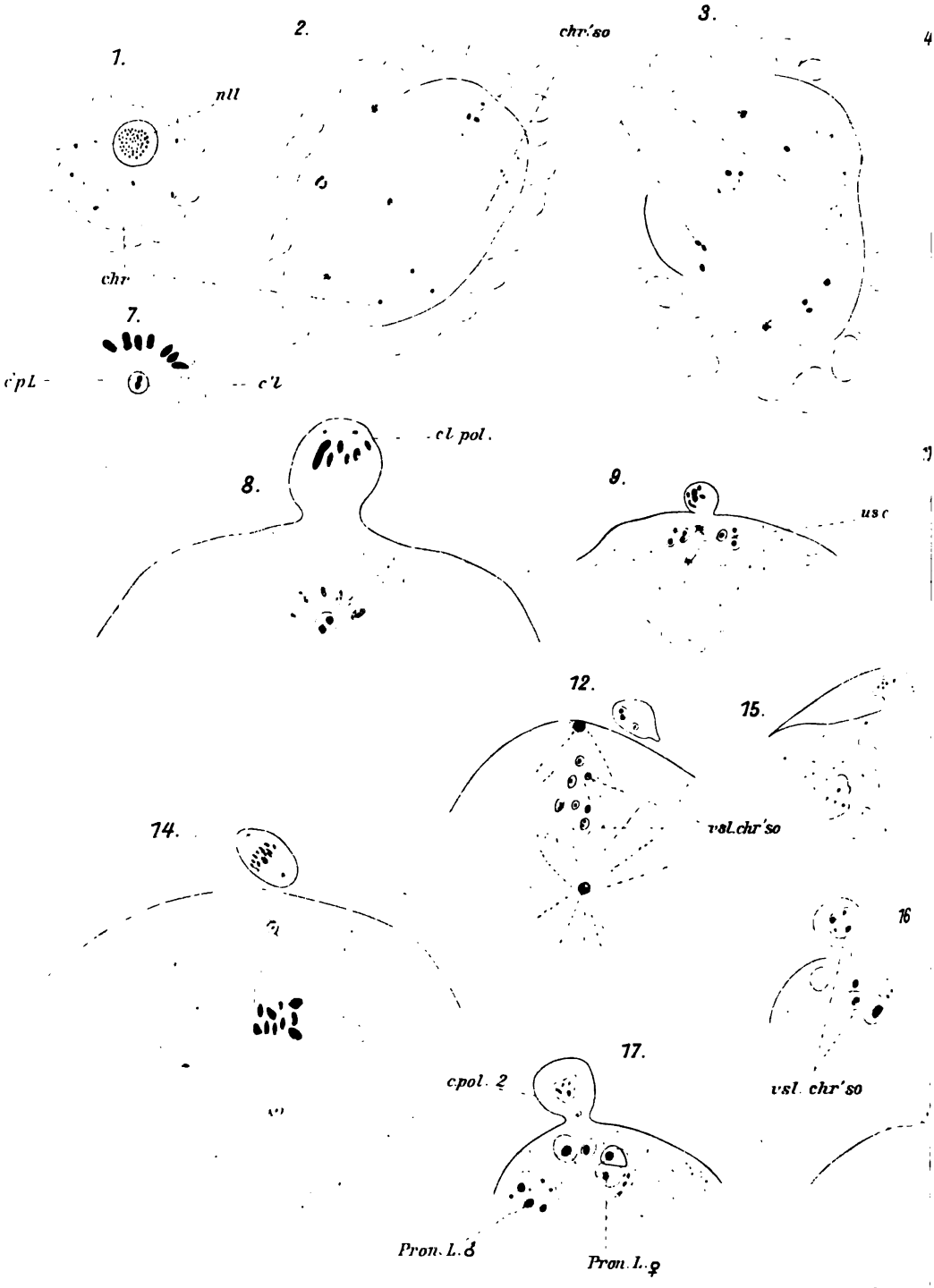
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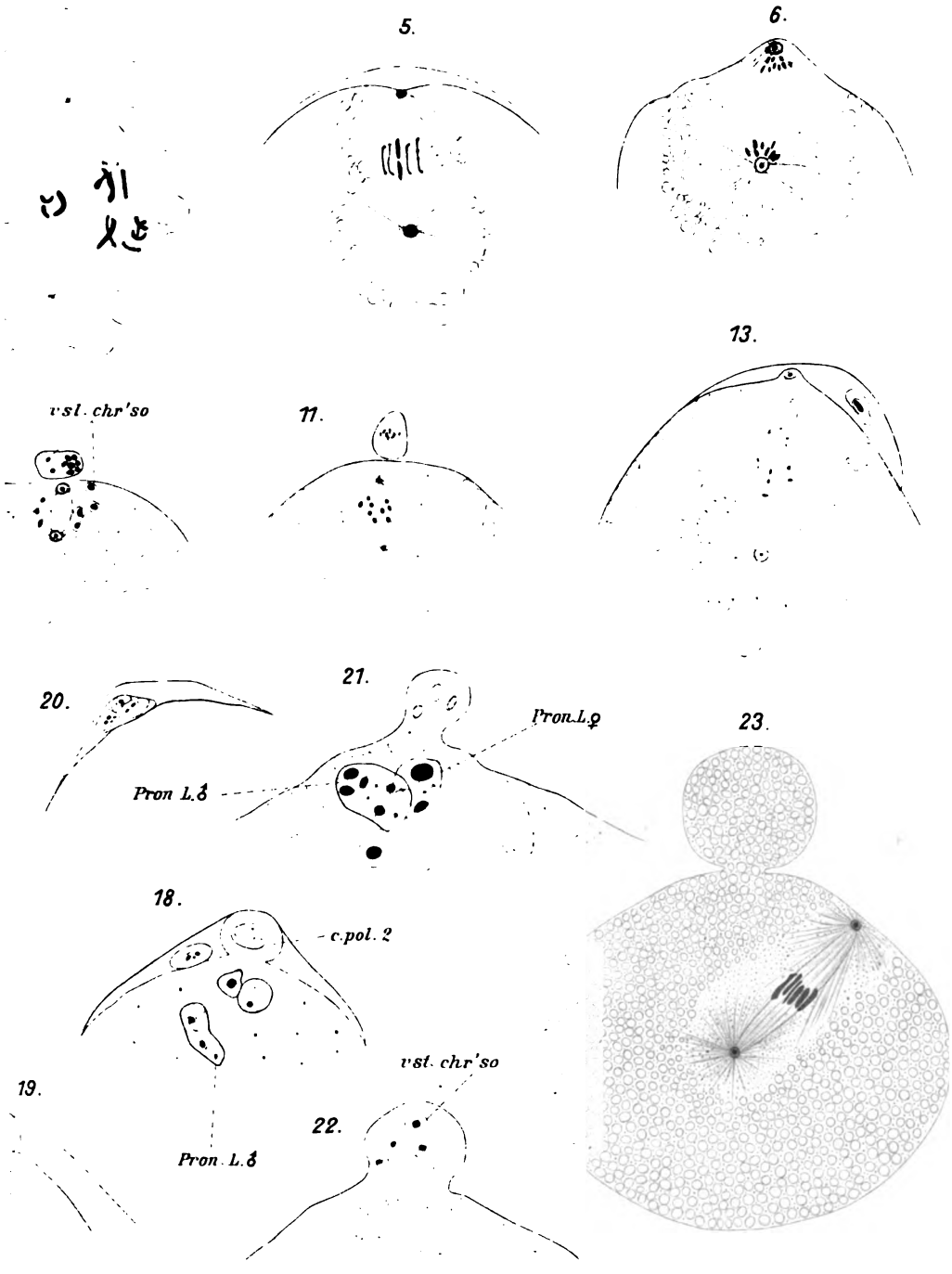

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[Reprinted from BIOLOGICAL BULLETIN, Vol. IX.. No. 1, June, 1905.]

THE EYE OF CRYPTOBRANCHUS.

ALBERT M. REESE.

Since no investigations, apparently, have been made on the anatomy of the eye of the American giant salamander, *C. alleghenïensis*, a brief description of that organ may be of interest.

In the living animal the eyes are quite inconspicuous, due to their small size, their lack of bright coloration, and to the wrinkled condition of the skin that surrounds them. The pupil, as seen from the surface, is very irregular in outline ; it appears as a small, jagged, black spot in the center of the gray iris. The eyes of the living *Cryptobranchus* do not differ markedly in appearance or relative size from those of *Necturus*.

In order to study their structure, the eyes, with a little of the surrounding tissue, were removed and sectioned in colloidin. Owing to the hardness of the lens it was not possible to cut very thin sections. The tissue was stained *in toto* with borax carmine, and after the sections were arranged serially on the slides they were stained with Lyon's blue. The memorandum as to fixation having been lost, it is not possible to give the method, but the results were fairly satisfactory except in the case of the layer of rods and cones of the retina. The figure represents a section through the middle region of the eye, passing through the optic nerve and the pupil.

The sclerotic coat (*Sc*) is rather indefinite in extent, and is largely chondrified. This cartilage forms a very thick capsule that surrounds considerably more than half of the globe of the eye. It is perforated, of course, at the back for the passage of the optic nerve. The walls of the cartilaginous capsule are not of homogeneous structure throughout, but are penetrated at places by ingrowths of tissue apparently derived from the choroid (*Ig*). The unchondrified portion of the sclerotic is, as has been said, of rather indefinite amount. It extends in front of the eye, between it and the superficial epithelium, to form a comparatively thick and, one would think not very transparent, cornea (*C*). In the fibrous portions of the sclerotic, as in other parts of the

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eye, are frequently seen large, black, many-branched pigment cells (*Ps*).

The corneal epithelium (*E*) is a direct continuation of the general epithelium of the head, there being no lids. As seen in the figure, the corneal epithelium is somewhat thinner than that of the rest of the head, and is composed of several layers of small cells with strongly-staining nuclei; the nuclei of the deepest layer are somewhat larger than the more superficial ones.

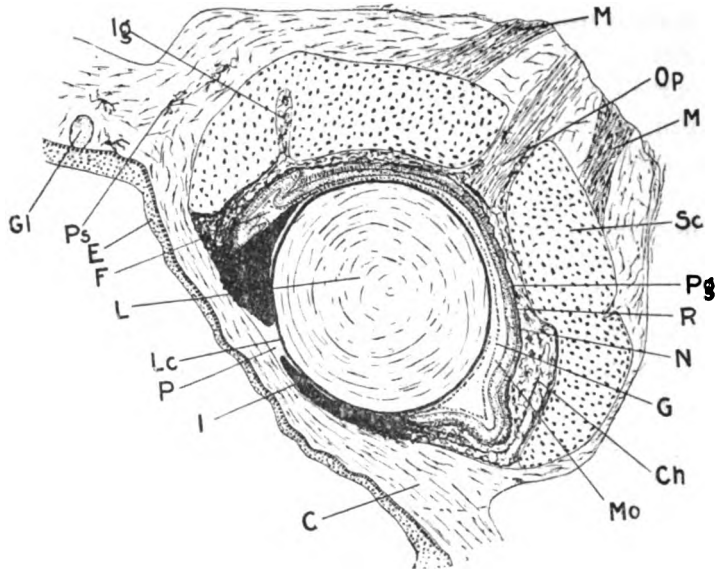

FIG. 1. Section through the eye of *Cryptobranchus Alleghenicnsis*. *C*, cornea; *Ch*, choroid; *E*, epithelium (conjunctiva); *F*, fold of the retina; G, ganglion-cell layer of retina; *Gl*, gland in the skin near the eye; *I*, iris; *Ig*, ingrowth of choroid into the cartilaginous sclerotic; *L*, lens; *Lc*, lens capsule; *M*, muscles; *Mo*, molecular layer of retina; *N*, inner and outer nuclear layers of retina; *Op*, optic nerve; *P*, pupil; *Ps*, pigmented layer of retina; *Ps*, branched pigment cells; *R*, layer of rods and cones of the retina; *Sc*, cartilaginous sclerotic.

In spite of the fact that the eye seems to have practically no power of motion, there are present the usual muscles, and they are of considerable size; two of them are shown in the figure (*M*).

The choroid (*Ch*) lies in immediate contact with the inner surface of the cartilaginous sclerotic, and is continued in between the front of the lens and the back of the cornea, in the usual

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way, to form the iris. It is more or less closely filled with the irregular, black pigment cells that were mentioned in connection with the sclerotic. These cells are seen along the tract of the optic nerve as it passes through the cartilaginous sclerotic. No distinction of layers can be made out in the choroid, nor can a true ciliary process be determined, though a thickening and folding in the region ordinarily occupied by that structure might be interpreted as a ciliary process; no connection of this thickening with the lens can be determined, however, and the appearance of a ciliary process seems to be largely caused by a curious folding of the retina, to be presently described. The reference line *Ch* ends in the thickening just described. In that portion of the choroid that extends in front of the lens to form the iris (*I*) the pigment cells increase to such an extent that the iris is almost or quite black, though this dark color is not so evident in the living eye.

The pupil (*P*) is, as has been said, small and irregular in outline. The retina, though not particularly well fixed in the material studied, shows at least six layers, which may be given the names usually applied to the corresponding structures in other eyes. The outermost (*Pg*) is the pigmented layer; it is comparatively thin, but shows an inner pigmented and an outer nuclear portion. Close to the inner surface of the pigmented layer, though often torn from it, is the layer of rods and cones (*R*); it has been called the layer of rods and cones, though, owing to imperfect fixation, the two structures are indistinguishable. Next to the layer of rods and cones lies a deeply-staining layer (*N*) that, in favorable sections, shows an indistinct division into an outer and an inner zone, which might be called the outer and inner nuclear layers, respectively. The two nuclear layers are composed of similar round, granular elements. Inside of the inner nuclear layer is a finely-granular, nonstaining layer which probably corresponds to the inner molecular layer (*Mo*). The outer molecular layer is not distinguishable in the material at hand. The innermost layer of the retina (*G*), which seems to correspond to the ganglion-cell layer, is composed of a single row of large, rounded or oval elements that stain deeply like the elements of the two nuclear layers. No layer of nerve fibers can

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be seen inside of this ganglion-cell layer, nor can the connection of the optic nerve (*Op*) with the retina be determined. The ganglion-cell layer lies, as a rule, in close contact with the lens, so that there is no vitreous cavity; the narrow space that sometimes appears between the inner layer of the retina and the capsule of the lens is probably due to a slight distortion of the eye.

One of the most striking features of this eye is the large surface covered by the retina; it extends as is partially shown on the right side of the figure, for some distance in front of the region of the ciliary process, if the thickening of the choroid may be so called. Another striking feature of the retina is the marked fold seen in the region of the ciliary thickening. All of the retinal layers take part in this fold (*F*), which varies somewhat in complexity in different eyes, but is evidently a normal condition and not a mere artifact.

The lens (*L*) presents no striking peculiarities; it is almost spherical in form, and completely fills the cup of the eye, so that the vitreous cavity, as has been said, is obliterated; a small space between the front of the lens and the cornea (at the end of the reference line *P*) may, perhaps, be taken to represent the aqueous cavity. The lens is surrounded by a comparatively thick capsule (*Lc*), whose distinctness is somewhat exaggerated in the figure.

The correlation of some of the above-described structures with the habits and mode of life of the giant salamander is not difficult to determine, but in other cases the correlation is not so certain.

The flattened anterior surface of the bulb, for example, is seen in most aquatic amphibia and may be merely a measure of protection against injury by coming in contact with the rocks and other objects under which the animal may hide; again a flattened or depressed cornea would evidently offer less friction in swimming.

A cartilaginous sclerotic is common among the amphibia, but it is difficult to see the necessity of such a heavily chondrified sclerotic in an eye that is so deeply buried as is this one. The spherical lens resembles the same structure in the teleosts, and probably indicates that the eye is especially adapted to vision at

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short range, the lack of transparency of the surrounding medium making long-range vision impossible, in any case. Although no definite experiments have been made along this line by the writer, it seems probable, from general observations, that *Cryptobranchus* is not very keen of sight even at short range.

The slight development of the ciliary process and the apparent absence of the ciliary muscles make it difficult to see how this animal can have any power of accommodation, since there is nothing that corresponds to the processus falciformis of the teleost eye. It is possible, therefore, that objects are clearly seen only when they are at a certain distance from the eye.

The unusually large extent of the retina may be a compensation for the slight power of motion possessed by the eye as a whole, so that the image of an object may fall upon a sensitive surface even though the object be without the ordinary line of vision.

The absence of the vitreous chamber may be correlated with the unusual refractive power of the lens, which makes a further refractive medium unnecessary, and necessitates the shortening of the eye-ball to bring the retinal surface to the focus of the highly-refractive lens.

SYRACUSE UNIVERSITY,
January 28, 1905.

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Adrenal Tumors in the Kidney of the Frog.

By W. M. Smallwood.

With 6 Figures.

While studying the frog in one of our courses this fall I noticed that the kidneys of one were much enlarged and resembled the normal kidneys in no respects excepting position. My first thought was that they might be parasitised or possibly exhibiting some phase of hermaphroditism. Fortunately the animal had just been killed and I was able to fix them at once.

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For fixing, CARNOY's fluid was used, the tissues being left in this mixture over night and then given a thorough washing in 95 % alcohol. I was not able to examine the tissues at once but found them to be in an excellent state of preservation after several weeks had elapsed.

The normal kidneys of the frog are about 14 mm long and 4 mm wide, rather flat, oval bodies lying in the posterior part of the coelome. In color they are dark red, the familiar color of the kidney. On the ventral surface of each there is an elongated, somewhat irregular band of tissue, the adrenal, which extends nearly the entire length of the kidney (Fig. 1). A cross section of the kidney shows that the adrenal varies in thickness being somewhat flat on the ventral surface and dipping into the kidney giving the adrenal a hemispherical shape. It is to be noted that the adrenal tissue occupies only a small part of the kidney.

Fig. 1. A normal sized kidney magnified 2 ×, showing the normal adrenal.

The investigations of BALFOUR ('81), MITSUKURI ('82), VINCENT ('97), WELDON ('84, '85) and others indicate very clearly that we are to find the origin of the adrenal bodies in Reptiles, Birds and Mammals in two distinct sources; the medullary substance is derived in part at least from the sympathetic nervous system, while the cortical substance arises either from the mesothelium or from the same cells that constitute the glomus of the pronephros. Similar results are at hand for fishes. While I did not find that the detailed origin of the adrenals had been worked out for Amphibians yet we may infer from the manner in which this body arises in all other Craniata that it probably has a similar origin in this phylum. So that it would not be very surprising if we should find a case where the adrenal tissue had grown down into the kidney, instead of being limited to the surface, and had produced a pathological condition. KELLY ('99, p. 283) in describing tumors in man makes mention of the frequent occurrence of accessory superadrenals, and further states that the cells of the adrenals become misplaced being found in the liver, solar plexus, epididymus etc. (cf. RIBBERT '04, p. 426).

In the kidneys under consideration (Figs. 2, 3) it is very evident that they have increased greatly in size being 21 mm long and 8 mm wide which is much larger than the normal kidney. This increase in size affects also the thickness as well. The color is whitish with very little evidence of any brownish pigment such as is found in similar tumors in the human kidney. Both kidneys have a large number of

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lobes having a generally similar shape but of different size. On the dorsal side of each no evidence was revealed, upon gross examination, of the presence of kidney tissue. On the ventral side of the left kidney there was a narrow band of kidney tissue (Fig. 3), the size

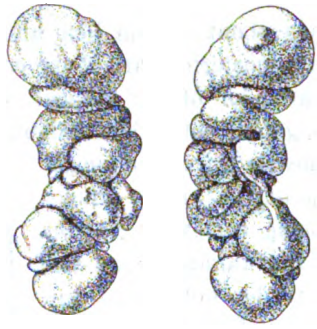

Fig. 2.

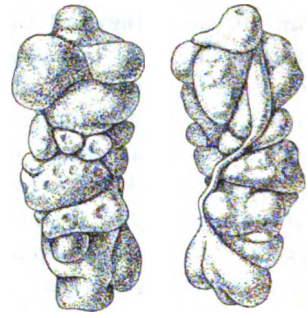

Fig. 3.

Fig. 2. The dorsal and ventral view of the right kidney having the adrenal tumor. Mangified 2 ×.

Fig. 3. The dorsal and ventral view of the left kidney having the adrenal tumor. Magnified 2 ×.

of the lobes concealing the rest. On the ventral side of the right kidney between the large lobes on each end there was considerable kidney tissue evident.

Neither in a gross study nor in a subsequent histological examination was there evidence of the normal adrenal in its normal position.

The urinogenital duct on the left side was on the inner border of the kidney instead of being on the outer border, its normal position. The attachment of this misplaced urinogenital duct would seem to indicate that it had been carried over the ventral surface of the kidney by the growth of the tumor because its suspensory membrane was attached along the ventral surface of the kidney.

A study of the cytology of these kidneys, using the well known basic and plasma stains, revealed the real character of the foreign growths. Sections were made through the various parts to determine whether all parts were in agreement with the result that the small and large lobes were found to exhibit the same structural characters. Fig. 4 shows that there are cavities of different sizes and that finger-like processes extend into them. Around the peripheral portion of the tumor the cavities are much smaller and in some instances none were observed. Each lobe is enclosed in a connective tissue capsule which separates the tumor from the remaining kidney tissue.

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The walls and solid portion of the tumor are composed of cuboidal epithelial cells the nuclei of which are very prominent. These cells are supported by a rich growth of stroma (connective tissue), which is equally evident in the branching tufts or finger-like processes. In the larger lobes there were found several places where numerous leucocytes (Fig. 4 *Leu*) were collected possibly indicating a state of inflammation.

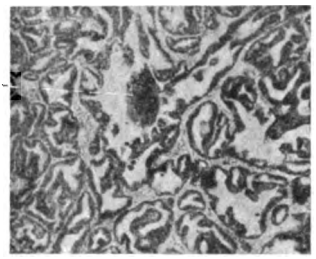

Fig. 4. A microphotograph of the adrenal tumor in the frog. *Leu* leucocytes.

Many of the nuclei were undergoing division mitotically but I was not able to find well formed centrosomes nor spindle fibres. The chromosomes exhibit a great variety of combinations. In one (Fig. 5) in the early anaphase there were 4 distinct chromosomes but there was also in the same field under the oil immersion lens a dividing cell in which I could not make out

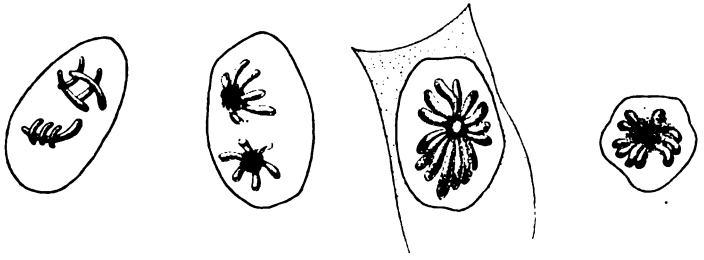

Fig. 5. Nuclei showing mitosis characteristic of this adrenal tumor.

any definite number of chromosomes. A careful study of the nuclei in various parts of the tumor revealed the fact that the cells were dividing rapidly but in nearly every instance atypically, i. e. the chromatin passed through various abnormal stages and it was impossible to classify them into any definite processes; for example in one cell there were undoubtedly 14 chromatic processes extending in all directions from a central mass of chromatin like a rosette while in others there were frequently many more.

Not only do these atypically dividing cells give rise to different forms of nuclei but also small bodies in the cytoplasm taking a basic stain are frequent (cf. YOUNG '97, p. 10) which are probably to be interpreted as chromatic masses that were not included within the nucleus as it assumed the so-called "resting state".

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Possibly associated with these chromatin masses in a causal relation, in part at least, are the various sized vacuoles (cf. GILCHRIST '96, p. 316) in the cytoplasm for it is well known that chromatin bodies are frequently found during maturation and early segmentation enclosed in the cytoplasm in vacuoles (LILLIE '02, p. 482; HARGITT '04, p. 461). The many examples of mitotically dividing cells and especially atypical cells is characteristic of tumors. No evidence of amitotic division was noted.

A comparison of the cells of the normal adrenal of the frog with those in the tumor showed that they were of the same lenght but somewhat wider and had more definite cell walls but aside from these two characters it was impossible to distinguish them. The lumen of the normal adrenal was relatively much smaller than in the tumor. The similarities of these tumor cells to the normal adrenal cells is so striking that one can not long be in doubt as to their being one and the same tissue. If this is the correct interpretation, then we have in the frog a case of an adrenal tumor developed from the entire adrenal body.

In conformation of the suggested origin of these tumors in the kidneys of the frog, we may refer to the following conclusions: BEARD ('03, p. 540) believes that "tumors are referable to abnormal attempts at development on the part of one or more vagrant or aberrant germ cells". While RIBBERT ('04, p. 426) states in describing the various adrenal tumors: „sie gehen überall aus isolierten Nebennierenteilen hervor", BASHFORD and MURRAY ('04, p. 413) conclude from their observations "that malignant new growths were virtually reproductive tissue arising in abnormal situations and possessed of an independence and power of growth like that of the testis in the Mammalian body".

An examination of the kidney tubules in general and a minute study of their individual cells in both the normal and tumorous kidney failed to reveal any constant differences. This would seem to indicate that, aside from a certain amount of atrophy due to the pressure of the tumor, the remaining kidney tissue was not structurally modified by this pathological condition.

A comparison of these observations with the well known adrenal tumors in the kidney of man shows many points of similarity. Such tumors in man may or may not conceal the normal kidney tissue on gross examination. Usually these adenomata are distinguished by a characteristic brownish color. DELAFIELD and PRUDDEN ('04, p. 644) distinguish papillary and alveolar adenomata and from their description of these two classes it is evident that these tumors in the frog show

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a general agreement with the papillary type. They further call attention to the fact that the stroma may be present in considerable quantities which is a very prominent feature of these tumors. On the other hand if these tumors are classified according to the plan offered in the syllabus of COUNCILMAN and MALLORY '04, they would be derived from the mesenchyma and designated as hypernephroma.

During the past few weeks there has come into my possession a slide of an adrenal tumor (human) of the papillary type which is almost identical with this tumor described above for the frog. The slide was loaned me by Dr. H. S. STEENSLAND, of the Syracuse Medical College, who has assisted me very materially in the interpretation of these pathological conditions. This human tumor is included within the kidney with no external evidence of its presence. The branching papillae arise from the walls of the alveoli and are covered with cuboidal epithelial cells. These branching tufts as well as the alveolar walls have a large amount of stroma. The lumina of the tumor are of irregular shape (Fig. 6).

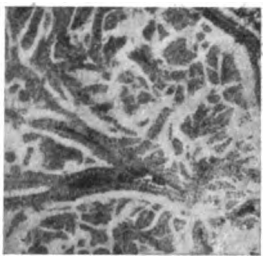

Fig. 6. A microphotograph of an adrenal tumor in the human kidney.

BASHFORD and MURRAY ('04) working in the Laboratory of the Cancer Research Fund of the Royal College of Physicians of London have accumulated a large number of specimens illustrating malignant new growths, not only in the domesticated animals but in many other vertebrates. They include 65 observations in their list showing tumors in all of the phyla from Pisces to and including Mammalia. But one observation is cited for Amphibia and that was a malignant cystic ademona in the testis of the Giant Salamander. The results described in this paper add one more observation to the Amphibian phylum and the conclusions reached are in admirable agreement with the following from the same authors: "The clinical, pathological, anatomical, and microscopical characters of these new growths are identical with those found in man in all essential features".

Zoological Department, Syracuse University, February 1, 1905.

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NOTES ON THE VARIATIONS OF RHEGMATODES.

CHAS. W. HARGITT.[1]

During the summer of 1900 I collected about two hundred specimens of *Rhegmatodes tenuis* for the purpose of studying its development. But this I was unable to carry out, owing to the inability of keeping the medusæ long enough in the aquarium to secure the eggs, few seeming to bear ripe gonads at the time of capture.

It was observed that many specimens showed more or less variability, and attention was then directed toward a study of the variations exhibited among the various organs. The comparatively few specimens obtained led to the postponement of a final study of the problem, hoping to secure additional material. This has been carefully sought during three subsequent summers, but in vain; only during 1902 have any specimens at all been found, and these very few and immature. The following "Notes" are therefore submitted as a slight contribution to the general problem of variation among Hydromedusæ, a more extended account of which was made by the writer in 1901.[2]

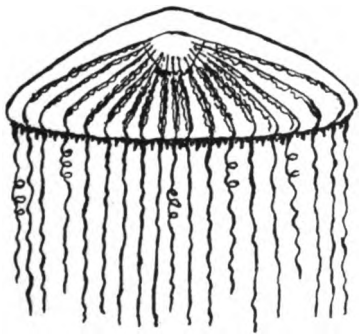

FIG. 1. *Rhegmatodes tenuis*. Semi-diagrammatic.

The general features of *Rhegmatodes* are shown in Fig. 1. The medusa varies in size at maturity from about 50 to 95 mm. in diameter. In younger stages the shape is more or less hemispherical, the tentacles and radial canals very much fewer in number, the earliest stage which I have found having but four canals and four tentacles. With growth the number increases very largely, but they are not correlated as at first, the latter becoming more numerous, besides also varying numbers of ten-

[1] Contributions from the Zoölogical Laboratory, Syracuse University.

[2] BIOL. BULL., Vol. II.

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tacular spurs or clubs between the bases of the fully developed ones.

In view of the constantly increasing number of these organs, which are the more prominent organs usually involved in variation, it might seem a very uncertain problem to undertake anything like a critical determination of the quantitive variations. So far as the merely numerical aspects of variation are concerned this is undoubtedly true, as will be seen. I may, therefore, say in the outset that, both in view of the doubt just expressed, as well as in the comparatively small number of specimens available, no attempt will be made to present anything like a statistical account. It will be the purpose, rather, to submit a brief account of variations of a qualititative, or substantive character.

So far as I am aware, the only account of variations in the Family Æquoridæ is that submitted by Claus,[1] concerning *Æquorea forskalii* in a sharp critique of Haeckel's multiplication of species, and enlargement of the scope of the family. Claus calls attention to the striking differences exhibited by young specimens as compared with adults, and also to the almost equally marked differences as to coloration found in specimens of various ages and sexes, as well as to the highly variable character of the number of canals, shape of mouth and oral arms under different conditions of contraction, etc.

In connection with his description of *Rhegmatodes floridana*, Dr. A. Agassiz has merely referred to the fact that in the Æquoridæ, "the chymiferous tubes especially are very irregularly formed. They are frequently added all on one side of the spherosome, and almost fully formed before they begin to develop in the other half."[2]

The matters involved in these observations, while both of importance, hardly touch the more fundamental problems of variation as now understood. The critique of Claus is eminently fitting as a protest against the unwarranted multiplicity of species based upon such flimsy characters as those cited. The matters referred to by Agassiz deal more directly with phases of development than with those of variation, though closely related thereto,

[1] Arbeit. Zoolog. Inst., Wien, 1881, p. 283.
[2] "North. Am. Acalephæ," 1865, p. 97.

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as will be seen in some of the illustrations of features to be described presently.

In view of the difficulty in accurately studying the otoliths of preserved specimens no attention has been paid to that feature, except incidentally. Something of a similar sort is true concerning the matter of variation in tentacles. They are so subject to mutilation and loss, to inequalities of regeneration, etc., that these also have not been given special attention in connection with this species. Chief attention has been directed to the radial canals, and to a less extent the gonads.

Asymmetry.

In a few cases marked asymmetry was apparent. In Fig. 2 is shown a condition found in several specimens. In the particular one from which this sketch was made the medusa had 24 radial canals, 12 of which were within a single quadrant of the umbrella, the others being about equally distributed over the other three fourths of the body. As will be observed, several of these canals were incomplete, as were also the associated gonads, the latter appearing to be either rudimentary or tending to degenerate. A somewhat similar condition is shown in Fig. 8; where in addition to the asymmetry, and incompleteness of several of the canals, there is also a fusion of the terminal portions of some of the canals.

The earlier citation of the account of Agassiz concerning the very "irregularly formed" condition of certain of these canals during development is significant in this connection. It would seem as if the condition involved in the case from which this illustration was drawn might be of a similar nature, though in the present instance, it must be remembered, that we are dealing with an adult and not a young specimen. It may not be improbable that in certain instances these apparent irregularities found during development may persist as permanent conditions in the adult.

Radial Canals.

Attention has been already directed to the fact that the canals increase in number with the growth and age of the medusa. No attention need be taken therefore of this feature in relation to

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variation, though when it is remembered that in the vast majority of all Hydromedusæ the radial canals are normally but four in number, the question naturally arises as to the conditions under which this extraordinary increase has arisen, and what it may signify. Such an inquiry would, however, lead too far afield for our present purpose. Mayer has raised and briefly discussed

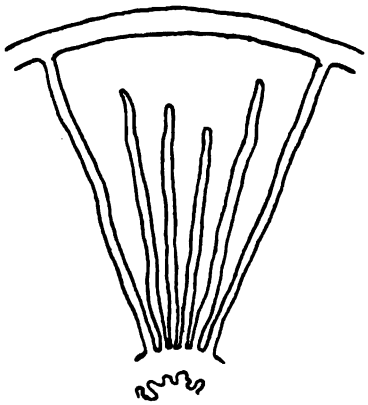

FIG. 2. Showing series of imperfect canals.

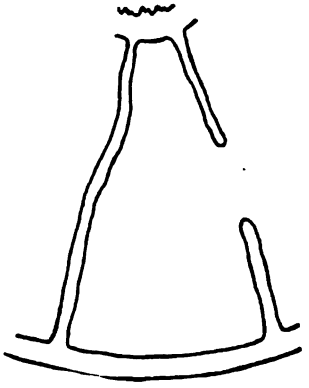

FIG. 3. Showing central and peripheral development of radial canal.

the problem in connection with a study of "The Variations of a Newly Arisen Species of Medusa."[1] In this paper Mayer suggests that one of the ways in which a pentamerous medusa may may have arisen from an ordinary tetramerous form is by bifurcation of one of the canals, which, if it became hereditary, would easily account for the condition of pentamerism.

The present writer has discussed somewhat the same general problem in an earlier paper (*op. cit.*, p. 239). If one had only a pentamerous or hexamerous condition for which to find some simple explanation, that just suggested might appear adequate. But when we have conditions like that of *Rhegmatodes*, not to mention many others of a similar sort, it will be more or less clear that such an account would be inadequate. It would seem far more probable that these supernumerary canals have arisen under the appropriate stimulus entirely independent of any special phyletic relations.

[1] "Variations in a Newly Arisen Medusa." *Bull. Brooklyn Inst. Arts and Sciences*, Vol. I.

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In Figs. 3–5, as well as in those already cited, are shown interesting and more or less common forms of variant canals. Similar conditions are also to be distinguished in several of the other figures given. For convenience we may discuss these features under the following heads: (1) Bifurcations, (2) loops, (3) spurs, (4) anastomoses.

It may be altogether probable that these conditions graduate somewhat insensibly into each other, so that they may really be but varying expressions of one and the same fundamental process But as suggested, such a classification will at least serve as a convenience in description.

1. *Bifurcations.* — Several illustrations of this feature have already been cited. Others are shown in Figs. 4–6. For the most part these apparently arise as a result of division of the peripherally developing canal, though the centripetally directed type is not lacking, as is seen in Fig. 4. It may be said in passing. that the figures given are for the most part typical ones, each representing usually several similar cases. In development the

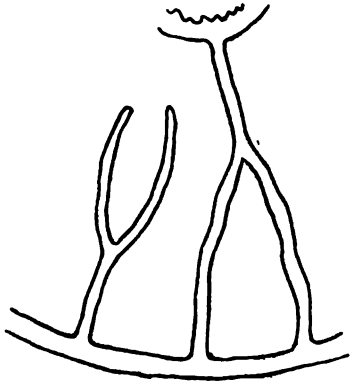

FIG. 4. Showing centripetal and centrifugal canals with bifurcations.

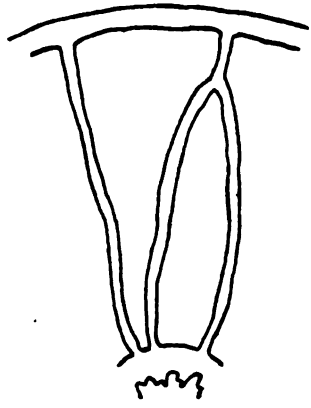

FIG. 5. Showing union of peripheral portions of two canals.

canals usually arise at the gastric pouch and grow toward the periphery. Agassiz (*op. cit.*) has said upon this point, "Additional chymiferous tubes in the Æquoridæ are developed from the digestive cavity, as shown by Kolliker, and not from the vertical tube, as in the case of the branching tubes of *Willia.*" This I have found to be the case in a study of the very young medusa. On

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the other hand, it must not be overlooked that in the Geryonidæ we have for the most part the opposite process, namely, the tubes arising from the marginal canal and developing centrally. Then in Figs. 4, 5 and 6, we have conditions which seem to show undoubtedly that such has been the mode of origin and development in *Rhegmatodes*. It brings before us, therefore, a form of variation at once morphological and physiological, or perhaps better, embryological.

2. *Loops.* — In Figs. 7 and 8 are shown what has seemed best described as loops. These seem to have resulted from the fusion of two or more canals whose terminal portions have approximated each other and finally become confluent. These were more or less common conditions among these medusæ. In

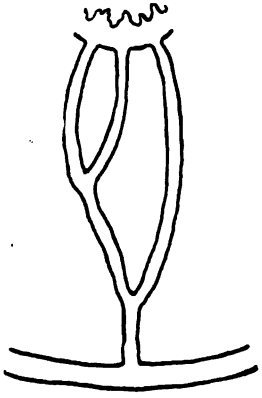

FIG. 6. Showing varying anastomoses.

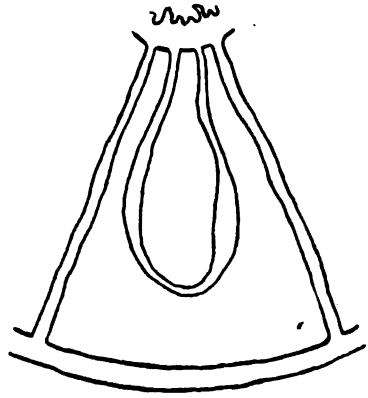

FIG. 7. Showing loop resulting from confluence of distal portions of adjacent canals.

my earlier paper (*op. cit.*) I have figured and described very similar features in *Gonionemus*, and Agassiz and Woodworth have likewise described similar features in the genus *Eucope*.[1] I incline to doubt whether any particular significance may be said to attach to such variations as these. It is certainly hard to discern any particular selective value they might have in the betterment of the species. It seems rather to be illustrative of those types of indefinite or fluctuating variations which abound more or less throughout the organic world, but having little or no bearing or significance in natural selection.

[1] "Variations in the Genus *Eucope*," *Bul. Mus. Comp. Zool.*, Vol. XXX.

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3. *Spurs.* — These features are among the most common of the variant phenomena associated with the radial canals. Very few medusæ fail to exhibit something of the sort in greater or less degree. They are, however, more than usually abundant in *Rhegmatodes.* In a forthcoming paper on "Variations Among the Scyphomedusæ"[1] I call attention to similar conditions associated with the abundant anastomoses which occur in species of *Aurelia.* As shown in several of the accompanying figures, especially 9 to 12, they probably have some such relation in *Rhegmatodes.*

4. *Anastomoses.* — These are features, as just suggested, involving to some extent the preceding, and almost as common, especially in *Rhegmatodes.* Figs. 10 to 14 are a few illustrations of a condition found in many specimens, both small and large. Whether a similar condition may be found to occur in other Æquoridæ I do not know, or whether similar features exist in other of the Hydromedusæ having supernumerary canals is apparently unknown. When compared with the complex canal system found in the genus *Aurelia* it would at once suggest the query as to whether it might imply any phyletic relation with these medusæ. But this does not seem to me to be the case. It has probably only a more or less indefinite correspondence in the most general way, barely enough likeness to suggest a remote parallelism, but nothing of greater significance. Furthermore, as suggested in the previous section, it is difficult to perceive anything like a definite variation in a given direction, tending toward the establishment of a type of hydromedusa with a complicated and correlated canal system such as that of *Aurelia.* And while the total of these variational features reached as high as 27 per cent., which is but slightly higher

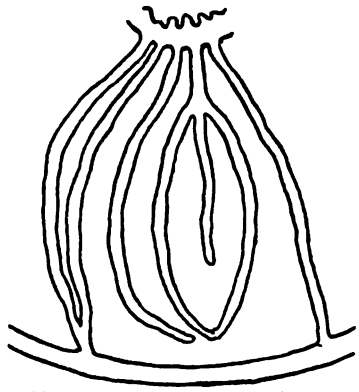

FIG. 8. Showing variously branched and united canals.

[1] "Variations Among the Scyphomedusæ," *Jour. Exp. Zool.*, Vol. II.

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than the ratio I have found in *Aurelia* (*op. cit.*), it may still be doubted whether it sustains any direct selectional value to the species, any more than in the case of *Aurelia*.

GONADS.

The gonads are closely correlated with the radial canals, forming a double fold along the line of each canal. In not a few cases it was found that the gonads of certain canals were more or less rudimentary. Whether this may have been due to arrested development, or to some wholly unrecognized cause it is impossible to say. As a rule, it may be said that in those canals marked by incompleteness, as in Figs. 2, 3 and 4, the gonads were of this character, *i. e.*, showed signs of arrested development, or atrophy. An apparent exception was observed in the case shown in Fig. 7, where in the loops formed the gonads were

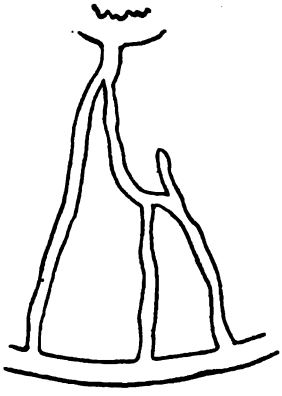

FIG. 9. Showing variations in branching, etc.

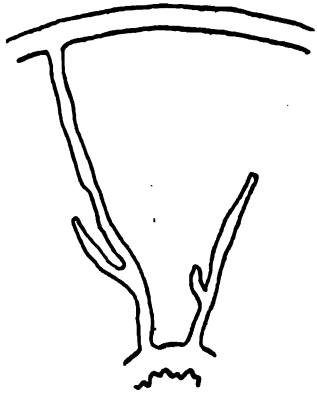

FIG. 10. Showing spurs.

larger and better developed than those of the normal canals of the same specimen. I think an explanation of this condition may be found in the fact that here the short loops of the canals were in closer connection with the gastric pouch, and thus provided with a larger food supply than in either of those of such incompleteness as shown in Figs. 2–4, or indeed the normal canals. Aside from these rather incidental aspects of variation there was little to call for special emphasis, or fuller details.

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TENTACLES.

As intimated in the outstart, there has been given but slight attention to variations in these organs. The tentacles of *Rhegmatodes* are both numerous and extremely delicate. It is therefore, difficult to distinguish numerical variations, or irregularity in position. I shall call attention only to one feature, concerning which, however, there is little to be said, the occurrence of branching, or bifurcation. This is a fairly common feature in many medusæ, and in my earlier paper I have figured and described numerous cases. Agassiz and Woodworth (*op. cit.*), have also described several cases among the Eucopidæ. Though somewhat careful attention was directed to this in *Rhegmatodes* anything of the sort is extremely rare. Only a single instance

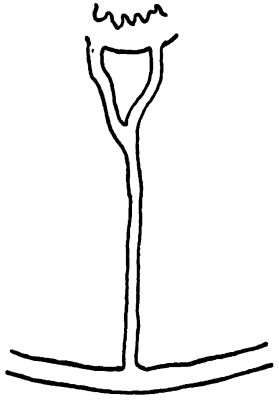

FIG. 11. Showing union of basal canals in a single one.

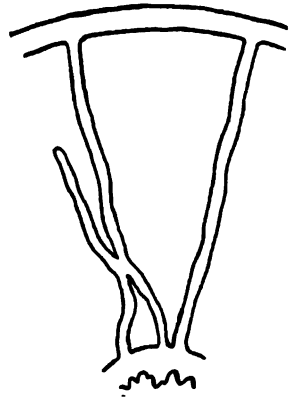

FIG. 12. Showing anastomosis and imperfect canal.

of what seemed to be a basal bifurcation of a tentacle was detected, and that was not so evident as to be absolutely certain.

As a final word upon this feature it may be stated that as a rule the number of tentacles was usually greater as the specimens increase in size. To this, however, there were many exceptions. For example, one specimen measuring 47 mm. in diameter had 66 tentacles, while another which measured but 36 mm. in diameter had 69 tentacles. This may be taken as somewhat typical of many similar cases. In the paper of 1901, already cited, I have called attention to numerous instances of a

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similar sort in *Gonionemus*. But as said in another connection, I do not attribute any particular significance to this feature in species like these, in which the numbers are large.

Otocysts.

The limited attention directed to these organs gave very much the same results as in the case of tentacles. Ordinarily they are said to occur in a more or less regular order between the bases

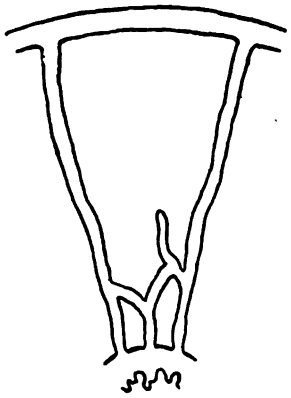

FIG. 13. Showing anastomoses.

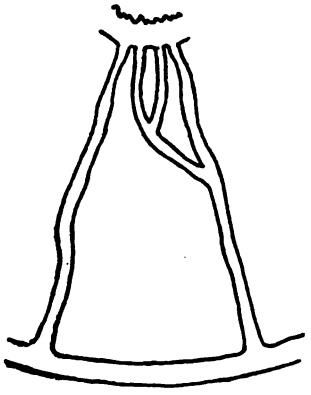

FIG. 14. Showing loops and anastomoses.

of the tentacles; and in some species this order is very exact, for the most part. But in *Rhegmatodes*, as in *Gonionemus* (*op. cit.*), this does not seem to be the case. While difficult in preserved material to determine in many cases the presence or absence of these minute bodies with exactness, still a careful search in many of the most favorable specimens failed to detect anything like a definite and symmetrical distribution of them. Agassiz (*op. cit.*) has described a single one between the bases of contiguous tentacles. In many cases two were found, and in a few cases even more. But as intimated above, both the difficulty of their determination, and the great liability of loss of tentacles make the matter of a dogmatic statement on this point unwarranted.

SYRACUSE UNIVERSITY,
September 9, 1905.

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VARIATIONS AMONG SCYPHOMEDUSÆ.[1]

BY

CHAS. W. HARGITT.

WITH 1 PLATE AND 17 FIGURES IN THE TEXT.

During the course of a study of variations among Hydromedusæ in 1901 the present writer became interested in facts of a similar sort which came under observation incidentally as certain specimens of various Scyphomedusæ were observed. Furthermore, during the course of extended studies in the development of Cyanea additional facts of a very interesting kind were observed. Still later in connection with experiments on regeneration in Rhizostoma pulmo ('04) other facts bearing more or less directly upon the same general problem were accumulated.

It is the purpose of the present paper to present a synopsis of the facts and to attempt a statistical exhibit of certain features of the variations observed, as well as an analysis of the data with a view to determine something of their bearings on the general problem of adaptation.

MATERIAL.

Most of the material was obtained at Woods Hole during the summers of 1901, 1902, 1905. Most of the ephyræ were obtained in April and May 1902, and in March 1904. The adults were collected by the writer at various times during these years in part, and in part by Mr. Vinal N. Edwards, collector for the laboratory of the United States Fish Commission, for whose kindness in turning it over to me for investigation it is a pleasure to acknowledge my grateful obligations. I was also permitted to examine a collection of about two hundred specimens of Aurelia collected by Mr. Geo. M. Gray, curator of the Marine Biological Laboratory supply department, for which courtesy my thanks are due.

The material was preserved for the most part in 5 per cent formalin. That of my own collecting was preserved in formalin

[1]Contributions from the Zoölogical Laboratory, Syracuse University.

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after treatment with the chromic acid method suggested by Browne, to the excellent results of which I am glad to certify.

My thanks are due to my son, George T. Hargitt, who has drawn most of the diagram sketches.

AURELIA FLAVIDULA.

The general facts of variation, or abnormality, as formerly regarded, in species of Aurelia have long been known. It is no part of the purpose of this paper to review in detail the history of observations along this line, yet it may not be amiss to cite some few of the more noteworthy among them. In a recent paper, "Uber Hypomerie und Hypermerie bei Aurelia aurita Lem.," Ballowitz has given a brief summary of the more important literature.

Von Baer[1] seems to have been among the first to record observations upon the several numerical variations in Aurelia aurita, and to point out certain correlations noticed, as well as their absence in some cases. According to this author the variation was estimated to be about 10 per cent.

Of more critical character are the observations of Ehrenberg[2] in 1835. This naturalist in an extended paper "Uber die Acalephen des rothen Meeres, etc.," reports with considerable detail upon variations observed in this medusa, and illustrates by numerous figures the more conspicuous aspects of the problem. He was able to confirm the earlier observations of von Baer, and considerably to extend them. Among many thousands of specimens casually noticed, and several hundreds examined with care he records having seen but two specimens of octamerous division of the gonads and comparatively few having a three-, five- and six-merous character. He records a single specimen observed with but one circular gonad about the mouth, which he considered to have been the result of a fusion of three or six single organs, as there were several openings into the stomach. In a case having double gonads he considered the condition to be due to a similar fusion of six organs as there were six openings distinguished. Like von Baer, this observer also recognized a

[1]Uber Medusa aurita, Meckels Archiv f. Physiol., Bd. viii, 1823.

[2]Abhandl. d. Königl. Akad. Wissenschaften, Berlin, 1835, S. 199–204, 1837.

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more or less perfect correlation among the several variable organs of the medusa, but also cites and figures an exceptional case in Taf. II, Fig. 12, in which in a rare octamerous specimen there were found fourteen rhopalia and twenty-eight principal canals, instead of sixteen and thirty-two respectively, as required to complete the symmetry. According to this author the ratio of variation was *estimated* as about 10 per cent (though Agassiz in a following quotation seems to have overlooked this point in Ehrenberg's work). It is not specified as to whether this includes the totality of variations, or refers to those of the vegetative organs. If the *former* it was probably too low; if the *latter* probably too high, as will be seen in the following data:

Haeckel[1] has recorded numerous facts of variation among European species of Aurelia and suggested their significance in relation to problems of evolution, though in his earlier account no details were given. In a later contribution[2] he has, however, discussed the problem in much detail, not only in connection with the adults but also in relation to the embryonic development, from segmentation and gastrulation on through the several transitional stages—scyphostoma, strobila and ephyra—up to the adult, giving excellent figures and descriptions of typical examples.

This author strongly maintains that numerical variations sustain intimate relationships throughout the entire ontogeny, and quotes Claus as holding similar views. "Ich nehme mit Claus an, dass alle Zahlenabnormitäten der reifen Aurelia schon bei ihrer Ephyrula-larve auftreten, und dass diese letztere sie bereits von ihrer Scyphostoma-amme geerbt hat. Wenn also Scyphostoma nur 2 gegenständige Tæniolen besitzt, so zeigt ihre Ephyrula nur 2 gegenständige Filamenten und die reife Aurelia später nur 2 gegenständige Gonaden."

In connection with the recent interest in the general problems of variation several brief accounts have appeared in reference to this medusa, but only two have gone into any details as to facts, or undertaken any analysis of them, namely, Browne[3] who in several contributions has discussed with pains and ability a very large number of observations made upon both adult and ephyræ;

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[1]Das System der Medusen. Jena, 1879.

[2]Metagenesis und Hypogenesis von Aurelia aurita, S. 26. Jena, 1881.

[3]Quart. Journ. Mic. Soc., vol. xxxvii, pp. 245. Biometrika, vol. i, p. 90. 1901.

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and Ballowitz,[1] who has devoted attention chiefly to adults, giving excellent figures of noteworthy variations, and includes also a valuable review of literature.

In view of these rather extended observations on the part of European observers and the almost entire lack of similar study of American medusæ it has seemed to the writer for several years that a comparison of Aurelia flavidula with Aurelia aurita might afford valuable results. And with this in view the data presented in the following pages have been worked out at such intervals during the past three years as have been available. I regret very much that a larger number of adult specimens have not been available, the hope of securing which has delayed the final appearance of the paper. It is believed, however, that sufficient data are presented to show at least something of the extent and significance of the variations.

As already intimated, almost nothing along these lines has been attempted in relation to American Scyphomedusæ, while the summary of observations by the present writer is all that has been attempted on the Hydromedusæ. L. Agassiz[2] has left a few very brief and rather indefinite records of variation in Aurelia flavidula. Concerning numerical variation he says: "These variations in number arise from the interpolation of similar parts, or from the abortion of some of them. I have observed on our coast specimens with three, five, six and seven crescent-shaped bodies, and the number of indentations along the margin increased correspondingly. These deviations from the normal number are rare with our species, and though Ehrenberg does not allude to their frequency in the European, I should infer that they are more frequent in Aurelia aurita than in Aurelia flavidula, for the simple reason that malformations of the crescent-shaped bodies are rarely met with in our species."

As will be noted, there is apparent in these observations of Agassiz, the same general assumption of a more or less close correlation among the several organic systems of the medusæ. I am inclined to regard this as in some measure due to a temperamental predisposition on the part of these earlier observers, perhaps growing out of the peculiar ideas in reference to such matters

[1]Archiv. f. Entwickelungsmechanik der Organismen, Bd. viii, S. 239. 1898.

[2]Contr. Nat. Hist. U. S., 1862, vol. iv, p. 51.

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more or less prevalent at that time. Certain it is, that either the more recent work on these lines have been more critical and discriminating, or a remarkable change has taken place since the earlier records were made. Possibly something of both may be true, though I incline to regard the former as more probable.

My investigations have been directed chiefly to two series of facts, namely: Variations as exhibited in the ephyra, and those found in the adult of Aurelia flavidula. Incidentally I shall direct attention briefly to certain other species which have been studied in connection with those of Aurelia, though of these no details will be undertaken, since in only a few cases have sufficient numbers been examined to warrant any general conclusions.

Being strongly convinced of the general correctness of Haeckel's view as to the relations of variations of the adult to similar conditions found in the larva, or ephyra, it seemed desirable to secure collections of specimens from various localities differing more or less in physical conditions of environment in order to estimate the probable influence of such conditions in relation to variation. Accordingly I secured ephyræ from three localities adjacent to Woods Hole, in about equal numbers, approximately 500 from each. (Unfortunately I was unable to obtain adults from the same environments, since their locomotor powers, influence of currents, winds, etc., carrying them every whither, made this quite impracticable.)

As is very well known, the Discomedusæ are characterized in general by the octamerous lobing of the umbrellar margin, correlated with which are eight rhopalia, or sensory bodies; and by the tetramerous form of the stomach and oral arms. This is more particularly the case with the semostomous group, to which belong most of our larger medusæ, of which Aurelia and Cyanea are good examples.

As will be seen, therefore, the organization of these medusæ, leaving out of account the tentacles, which differ greatly in the several genera, presents two fairly differentiated and independent sets of organs, namely, the marginal or sensory, and the central or vegetative. In many cases these sets are correlated for nutritive purposes through the radial canal system, though of themselves they may for the present discussion be considered as independent systems, distinctively organized and definitely correlated, as

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indicated above. In keeping with this view we may naturally proceed upon our analysis and comparisons under two heads:

(1) The marginal system; and (2) the nutritive and reproductive, or vegetative system.

Concerning the canal system nothing will be said in connection with the study of the ephyræ, since during the early larval history this system is but slightly developed and therefore of but small consequence in relation to variation.

Furthermore, since as already suggested the purpose is in part a comparison of the aspects of variation presented by the ephyra and adult, we have again a two-fold division of the subject. And since in the order of nature the ephyra precedes the adult this may as well be taken as the order of research.

Variation in the Ephyra.

Aside from the investigations of Haeckel (*op. cit.*), so far as I am aware Browne is the only investigator who has taken up this phase of the subject in detail. And furthermore, since heretofore investigation has been directed almost wholly to European species it has seemed to me highly desirable that similar observations be made upon those of American waters, in order to have some broader basis of comparison and deduction.

Marginal Organs.

The ephyræ studied are of two series, first those collected in 1901–02, and second, those collected in 1904. I choose to consider them in this detached way chiefly from the fact that the latter were just in process of metamorphosis into young Medusæ, and it seemed better to study them with a view to securing possible evidence as to any ratio of selection which might be detected as occurring during this process. The ephyræ were all of Aurelia flavidula, except possibly a stray specimen of Cyanea which might have drifted among them. But these were exceedingly rare, if occurring at all, since an examination of several hundred of the series of 1904 in process of metamorphosis failed to reveal the presence of a single specimen. Moreover, since in an earlier study of the development of Cyanea I found abundant evidence of similar variation, the presence of an occasional specimen in the estimation of percentage variation could hardly affect the results.

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Of the first series a total of 1512 specimens were examined. Of this number 398 or 26 per cent showed variations in some one or more of the organs. Of specimens having less than the normal number of marginal organs there were but 19, or 1.25 per cent. Of those having more than the normal number of these organs there were 379, or 25 per cent. Details concerning these data are given in tabulated form in the following tables.

TABLE I.—*Showing Correlations of Marginal Lobes and Rhopalia.*

MARGINAL LOBES.	RHOPALIA.										
	5	6	7	8	9	10	11	12	13	14	TOTALS
5	1										1
6		1									1
7			15	13							28
8			2	1114	5						1121
9				7	206	4					217
10					2	84	1				87
11					1	1	28				30
12							3	13			16
13							1		8		9
14										2	2
TOTALS	1	1	17	1134	214	89	33	13	8	2	1512

Table I presents in graphic form the correlations existing between the marginal lobes and the rhopalia. In the vertical column at the left are given the number of marginal lobes ranging from 5 to 14, the smallest and largest number respectively found in any specimen. In the horizontal column at the top are

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given the number of rhopalia, while the number of specimens are arranged in the squares. While in general there is a very close correlation between these organs it will be seen that there are not infrequent departures from this rule, or in other words absence of correlation. For example, two specimens were found having only seven rhopalia while there were eight marginal lobes. Likewise there will be seen to be five specimens having more rhopalia than marginal lobes in normal octamerous individuals. A simi-

TABLE II.—*Showing Correlations of Oral and Gastric Lobes.*

GASTRIC LOBES.	ORAL LOBES. 2	3	4	5	6	7	TOTALS
2	1						1
3		1	3				4
4		2	474				476
5				1			1
6					3		3
7						1	1
TOTALS	1	3	477	1	3	1	486

lar condition of variation is shown in specimens having seven, nine, ten, eleven, and twelve marginal lobes and rhopalia.

A curve constructed by which to portray even more graphically the facts would involve the following factors:[1]

	MARGINAL LOBES.	RHOPALIA.
Mean variation	8.396	8.379
Standard deviation	.896	.872
Coëfficient of variability	1.06	1.04
Probable error of mean	± .015	± .002
Probable error of standard deviation	± .011	± .011
Average deviation	.626	.641

[1]For calculating the factors of this curve I am under obligations to my colleague, Dr. Smallwood.

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A study of the table will naturally give rise to the question as to how the several variations in these marginal organs are to be explained. For example it will be observed that twenty-three specimens had more rhopalia than marginal lobes. A reference to Figs. 1 to 3 will quickly afford an explanation of this phenomenon. The figures also show double and twin rhopalia, several cases of which were found. Similar cases are cited by Browne (*op. cit.*, p. 90), and in connection with their discussion he ventures the suggestion that perhaps in later development and during metamorphosis, by a growth of the margin these so-called twin rhopalia may become separated thus giving rise to an independent lobe. This seems to me to be extremely doubtful. As a matter of fact it is well known that during growth following metamor-

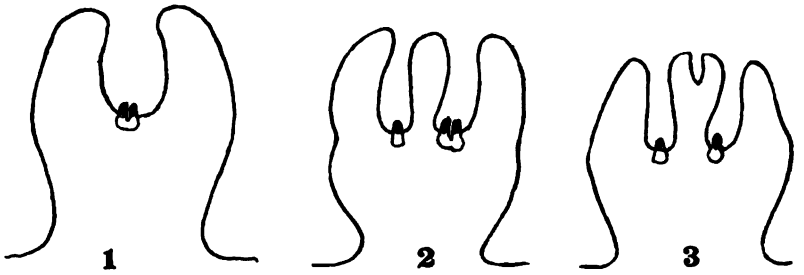

Fig. 1. Diagram of marginal lobe showing twin rhopalia.
Fig. 2. Compound marginal lobe, one of which contains twin rhopalia.
Fig. 3. Compound marginal lobes the central one notched at the tip.

phosis, increase of the marginal dimension takes place entirely by growth of the inter-rhopalial areas. This is very easily seen by a study of the appearance of new marginal tentacles and by the origin and multiplication of the branching canals. It may therefore be accepted as practically certain that the twin rhopalia of the ephyra continue such in the adult medusa. It is also almost certain that a similar condition is involved in the case of such double rhopalia as are shown in Figs. 3 and 4. In these respects, as in others, as cited by Haeckel (*op. cit.*) we may, I believe, regard it as undoubtedly true that the larval variations are carried over into the adult through the several phases of metamorphosis. Furthermore, this view is confirmed by the facts clearly established, that the ratio of variation found in the adults is essentially the same as that found in the ephyræ.

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As suggested above, it seems reasonably clear that the excess of rhopalia may be accounted for in the manner already proposed. But it remains to consider those cases in which the number of marginal lobes is in excess of the number of rhopalia. Of these there were found seventeen cases, as against twenty-three of the former. It is quite obvious that for these a different explanation must be found. We are here limited to two alternatives, namely, either there are cases in which for some reason there has been a failure of a given lobe to develop its usual organ; or on the other hand there may possibly be a subsequent and independent origin of an extra lobe. While I have found undoubted cases of the occurrence of the former condition, and am constrained to regard it as the more usual and probable explanation, at the same time I have found an occasional case in which a belated lobe appears to arise after the ephyra has become free from the strobila, but at the same time it must be admitted that in these cases there is usually found the accompanying rhopalium, though this is not always true. I am therefore constrained to consider both alternatives as possible, though giving to the first the larger probability.

A few unusual features in the marginal and rhopalial variations call for a merely passing notice. Fig. 1, showing an ordinary twin rhopalium, calls for little note aside from the statement of fact that it plainly occupies the position and relation of a typical organ, namely, the terminus of a single canal. And in this connection it may be well to recall that in the early ephyra-life all the canals are simple, that is, unbranched. It is only during the progress of metamorphosis that the complexity of the adult canal system is gradually differentiated.

Figs. 2, 3, show a series of extremely interesting variations of graduated complexity. The first shows a trifid condition of the otherwise normal ephyra lobe, though with the added abnormality of twin rhopalia in one of the notches. In the second there is shown a quadrifid lobe, the lappets of the median pair being small and not particularly remarkable, while in each notch of the outer lappets is a normal sensory body. Several other figures show similar features.

Among 486 ephyræ taken in the "eel pond," Woods Hole, in April, 1902, the variants numbered 144, or 29.6 per cent.

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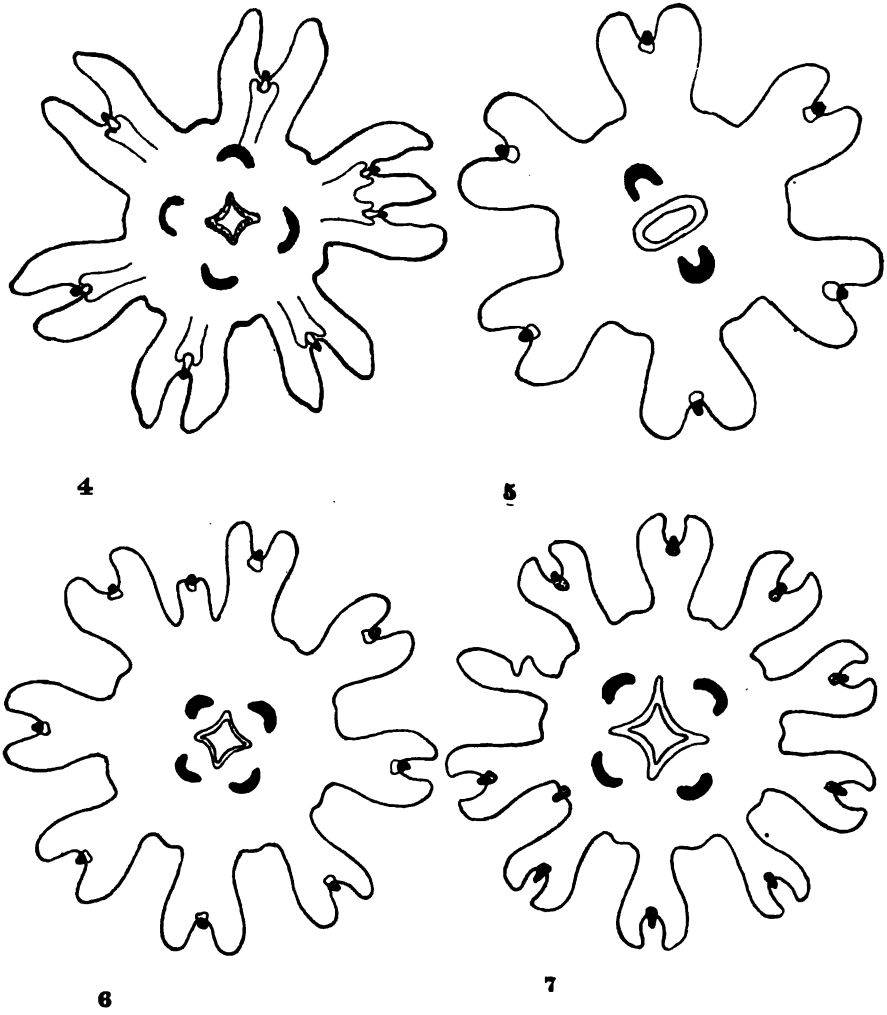

Fig. 4. Hexamerous ephyra, one lobe of which is compound.

Fig. 5. Hexamerous ephyra, with two-lobed mouth, and two gastric lobes.

Fig. 6. Ephyra with small supernumerary rhopalium, several examples of which were found.

Fig. 7. Ephyra with an adradial rhopalium, and one of the normal lobes devoid of an organ, perhaps due to injury.

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TABLE III.

No. Specimens.	Gastric Lobes.	Oral Lobes.	Rhopalia.	Marginal Lobes.
1	2	2	6	6
1	3	3	7	7
1	3	4	7	7
2	3	4	9	9
1	4	3	10	10
1	4	3	12	12
78	4	4	9	9
1	4	4	8	9
35	4	4	10	10
2	4	4	9	10
8	4	4	11	11
7	4	4	12	12
1	5	5	10	10
1	6	6	11	11
1	6	6	12	12
1	6	6	13	13
1	4	4	14	14
1	7	7	10	10

Each of these rhopalia occupied a single octant of the ephyra margin, and differed but little in size from the others. It should be stated that each was found on a different specimen.

In two specimens were found a very rare feature among these varied rhopalial phenomena, namely, the presence of a rhopalium in an interlobular, or adradial position, as shown in Figs. 6, 7. One of such cases I also discovered in connection with the study of the development of Cyanea. Here we probably have the origin of the condition which eventuates in the equally rare occurrence of an adradial rhopalium in the adult medusa, cases of which will be considered in a later connection.

Oral and Gastric Organs.

As compared with the marginal system that of the vegetative shows comparatively little variation, at least in the ephyra stage, though the present data are far from complete. In the first place

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the number of specimens tabulated was less than one-third of the entire number in the preceding series. This is due in part to the poorly differentiated stage of these organs in the early ephyra, the gonads being entirely lacking, and the mouth-lobes being often so contracted as to render certain determination impossible.

In Table II is shown the range of variation so far as accurate data are at command, including as in Table I divergencies or lack of correlation between the two sets of organs.

As will be noted, out of a total of 486 specimens, only 12 or 2.68 per cent vary from the normal.

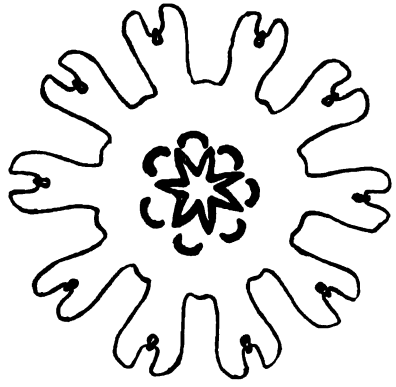

Fig. 8. Ephyra with seven gastric and oral lobes, and ten marginal lobes.

In several figures are shown illustrations of these aspects of our subject. In Fig. 5 is shown a sketch of one of these, in which there are but two oral divisions and two gastric pouches. One might suppose the directly opposite relations of these organs as figured to be unusual, but when compared with Figs. 6 and 7 it will be seen to be quite in keeping with that found in almost every case, that is, the angles of the mouth correspond with those of the stomach so that the pouches of the latter of course occupy intermediate positions. In other words, the angles of the mouth occupy the perradii of the body while the gastric pouches or lobes occupy the interradii.

A comparison of figures will readily show the lack of correlation of these organs with those of the marginal system, and at the same time the close correlations between themselves, the latter being likewise evident in the data of the table.

Additional data of a similar sort will be presented in connection with the second series, a comparison of which will still further emphasize the small ratio of variation as compared with that of the marginal organs.

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A Comparison of the Variations Exhibited by Ephyræ During Metamorphosis.

During the current summer, 1905, I was able to secure a collection of about 1000 ephyræ from Waquoit Bay, a body of water some ten miles east of Woods Hole, from which had also been secured a portion of the previous series in 1901. Among these were found 392 specimens which were just emerging into young medusæ. They varied in size from 7 to 14 mm. in diameter, the radial canals were well differentiated, and the gastric pouches easily distinguishable. There were also 218 ephyræ among the number which were entirely devoid of any indications of metamorphosis, indeed, apparently but recently escaped from the strobila. I was particularly glad to have an opportunity to study a series of this character from a strictly local environment and from the same brood, so to speak, since it afforded an opportunity to test a feature of variation already referred to, namely, whether varietal features existing in one stage are carried over into another, or whether during a period of metamorphism there was at work any selective processes.

While the numbers examined in these cases are too small to afford conclusive data on a problem of this character, they may at any rate afford a fair indication as to probabilities, and when taken in comparison with similar series in larger numbers, as in the former case, and also in connection with the observations of Browne (*op. cit.*), they may become correspondingly more convincing.

A comparison of the data presented in Tables IV and V will show, both in relation to the percentage variation and to the question of the persistence of varietal features during the several phases of metamorphism, rather striking points of likeness. So far as the ratio of variation is concerned it will be seen at a glance that it is so nearly the same in the several cases as to preclude the probability of anything more than slight and incidental differences. For example, in Table I the total per cent of variation is 26; in Table V it is 22.2; while in Table IV it is 22.9. Compared with the ratio obtained by Browne, which for 359 ephyræ taken in 1893 was 22.6, and for 1116 specimens taken in 1894 was 20.9, the results become still more conclusive.

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Of 218 ephyræ taken at Waquoit Bay in April, 1904, there were 50 variants, or 22.9 per cent. The chief features are tabulated as follows:

TABLE IV.

No. of Specimens.	Gastric Lobes.	Oral Lobes.	Rhopalia.	Marginal Lobes.
1	3	3	6	6
1	3	3	7	7
2	4	4	6	6
2	4	4	7	7
31	4	4	9	9
4	4	4	10	10
1	4	4	11	11
1	4	4	12	12
1	5	5	11	11
1	5	5	12	12
1	6	3	12	12
3	6	6	12	12
1	6	7	12	12

Now if we compare these results with those shown in Tables VI and VII, in which are presented in detail the variations found in 226 specimens of young Aurelia flavidula taken at Waquoit Bay in May, 1905, and in a collection of adults made at New Bedford about the same time it will be seen that they all tend to confirm the general propositions under consideration, namely, that varietal features found in the ephyra persist in the adult, and furthermore, that there is no evidence of any selective process involved during these several changes in ontogeny.

Of 392 ephyræ in process of metamorphosis, taken at Waquoit Bay in April, 1904, there were 87 specimens, or 22.2 per cent., which showed various aspects of variation, the principal features of which are classified in the following tabulated form:

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TABLE V.

No. of Specimens.	Gastric Lobes.	Oral Lobes.	Rhopalia.	Canal System.
1	2	2	6	Perradial ..... 0 1 1 1 Interradial 0 1 1 1
1	3	4	7	Perradial ..... 1 1 1 1 Interradial 0 1 1 1
2	4	4	7	Perradial ..... 0 1 1 1 Interradial 1 1 1 1
1	4	4	7	Perradial ..... 1 1 1 1 Interradial 0 1 1 1
45	4	4	9	Perradial ..... 2 1 1 1 Interradial 1 1 1 1
11	4	4	10	Perradial ..... 2 2 1 1 Interradial 1 1 1 1
4	4	4	10	Perradial ..... 2 1 2 1 Interradial 1 1 1 1
1	4	4	10	Perradial ..... 3 1 1 1 Interradial 1 1 1 1
3	4	4	11	Perradial ..... 2 2 2 1 Interradial 1 1 1 1
1	4	4	11	Perradial ..... 2 2 1 1 Interradial 1 1 1 1
1	4	4	12	Perradial ..... 2 2 2 2 Interradial 1 1 1 1
1	4	4	13	Perradial ..... 2 2 2 2 Interradial 2 1 1 1
1	4	4	15	Perradial ..... 3 3 3 2 Interradial 1 1 1 1
1	6	5	11	Perradial ..... 2 1 1 1 1 Interradial 1 1 1 1 1
6	6	6	11	Perradial ..... 1 1 1 1 1 1 Interradial 0 1 1 1 1 1
2	6	6	12	Perradial ..... 1 1 1 1 1 1 Interradial 1 1 1 1 1 1
1	6	6	13	Perradial ..... 1 1 1 1 1 1 Interradial 2 1 1 1 1 1
1	6	5	13	Perradial ..... 2 1 1 1 1 1 Interradial 1 1 1 1 1 1
1	6	6	13	*Ibid.*
1	6	8 (double)	15	*Cf.* Fig. 10.
1	8	8	13	Perradial .....0 1 1 1 1 1 1 1 Interradial 0 0 1 1 1 1 1 1

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Variations in the Adults.

As in the study of the ephyræ attention was directed chiefly to the marginal or sensory bodies, and to the central or vegetative, so likewise in the study of the adults the same systems have received primary consideration, though in the latter including also the canal system, as a correlating medium between the others. Attention was also directed to the problem of the probable influence of local conditions in determining variations. As favorable localities from which to secure specimens more or less subject to a definite environment, New Bedford harbor and Waquoit Bay were selected, the latter serving moreover, the further end of ascertaining the variations exhibited by ephyræ and adults under the same environment.

In addition to authorities cited in a previous part of this paper attention may be directed to the observations of Bateson and Romanes on the variations of Aurelia aurita. The latter has described in some detail variations found in this medusa and has illustrated by diagrams many of the features described. In both the illustrations and the analysis of the facts there is an apparent effort of the author to reduce the variations to as few symmetrical types as possible. As I have pointed out in another connection in reference to the work of Ehrenberg and Agassiz, these attempts to discover a law of symmetry, or perhaps better in modern phrase, a law of *regulation*, in the diverse variations encountered, have apparently been only partially justified. While it is doubtless true that in many cases, perhaps in a majority, some form of regulation may be distinguished, there are too many cases in which this is lacking to be considered as merely exceptions to such a law. A study of the following facts and illustrations, will I believe justify this view.

As Bateson in commenting upon Romanes' work has remarked, "It is impossible in regular threes, sixes, etc., to say that any particular segment is missing or added rather than another." And if this be the case with an organism like Aurelia, in which the several organs are so sharply differentiated as to be easily distinguished at a glance, it is much more likely to be true in organisms of more complex structure and less sharpness of differentiation.

In an attempt to ascertain the comparative frequency of certain

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variations in Aurelia, Bateson examined 1763 adult specimens taken on the Northumberland coast in 1892. In the tabulation of his results he presents details of only the gonads and oral lobes. Of these there were but 28 abnormal specimens, or a variation of only 1.6 per cent. Of the 28 abnormal individuals 19 he considers as "symmetrical varieties," and observes that the other 9 specimens, or 33 per cent are "irregular varieties" and are seen "for the most part in single specimens only." Here Bateson apparently falls into the same error which he has criticized in Romanes, namely, the attempt to reduce the variations to "symmetrical varieties," regarding "irregular varieties" as exceptional. But the presence of 33 per cent of the so-called "irregular varieties" is too large a proportion to be designated as exceptions.

As is well known Aurelia is an octamerous medusa, each octomere being characterized by a single, more or less dichotomously branched radial canal, at the terminus of the central stem of which is located the sensory body, or rhopalium, and separated from the adjacent octomere by an unbranched canal, as shown in several of the diagrams. In normal individuals this arrangement is very symmetrical, and easily distinguishable. It must not be inferred, however, that the several branching canals are exactly similar, or symmetrical. Indeed it may be safely said that probably no two in a given individual are exactly alike, any more than are two leaves of a given plant. Still, the differences are usually slight, striking variations occurring chiefly in those cases where departures from the typical arrangement are considerable. For convenience in following readily the subsequent discussions, it may be well to briefly remind the reader that for descriptive purposes the several canals have been designated by the special names, *perradial*, signifying those canals arising between the gastric pouches, or mouth angles; *interradial*, indicating those occupying intermediate positions, or emerging from the outer median portion of the gastric pouches; while the term *adradial* refers to the unbranched canals alternating with the other two series.

Gastric and Reproductive Organs.

Among the most conspicuous variations from the typical condition just described are those involving a numerical, or meristic departure. This will be readily understood when it is remembered

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that these organs are large and conspicuous, four in number, and usually the first to attract attention. It is doubtless on this account that so many of the earlier observations concerning variation in these medusæ dealt almost exclusively with this feature.

Among the commonest variation is the hexamerous form, where there are six each of the gastric lobes, gonads, and oral arms. This will be observed at a glance by comparing the several tables, especially Nos. IV and V. Next in frequency is the pentamerous type, where there is a symmetrical arrangement of the organs upon the plan of five. As the several details of these variations are specified so far as their numerical aspects are concerned it is only necessary to refer to the tables already cited. It may be well to notice briefly a few features not capable of tabulation. Among these are the not infrequent occurrence of signs of atrophy, as shown in Figs. 11 and 12. In the former it will be observed that associated with the small size of the pouch and gonad is the entire absence of the interradial canal and its marginal organ. In the latter will be observed the presence of a mere rudiment of a regressive gonad in one of the pouches, while in the opposite compound pouch there are two gonads, and in this case the absence of the perradial canal system.

Associated with variations in the number, is that of variation in the size and relations of the organs, as already pointed out in the figures cited. Attention was directed to the compound character of the organs. This is a very common occurrence, and probably is indicative of the manner of the origin of supernumerary organs of this character. However, the pentamerous and hexamerous condition is frequently distinguishable in the ephyra, and seem to be quite distinct from the beginning And I have found in Cyanea that occasionally trimerous polyps occur, and probably give rise to trimerous ephyræ and later trimerous medusæ. It may not be improbable that the suggestion of Ehrenberg (*op. cit.*), that the circular gonads which he observed were the result of fusion of what may have been earlier distinct organs, is quite as likely as that above. It may be suggested in this connection that I have never seen a case such as that cited by Ehrenberg, though its occurrence does not seem improbable, but in every case which has come under my observation of a compound gastric pouch, the gonads have been more or less distinct, as indicated in Fig. 12.

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Of 226 small adults collected at Waquoit Bay, May, 1905, there were 55 variants, or 24.3 per cent. The general features of variation are tabulated as follows:

TABLE VI.

No. of Specimens.	Gastric Lobes.	Oral Lobes.	Rhopalia.	Canal System.				
1	4	3	8	Perradial	1	1	1	1
				Interradial ...	1	1	1	1
1	4	4	7	Perradial	0	1	1	1
				Interradial ...	1	1	1	1
3	4	4	7	Perradial	1	1	1	1
				Interradial ...	0	1	1	1
26	4	4	9	Perradial	2	1	1	1
				Interradial ...	1	1	1	1
7	4	4	9	Perradial	1	1	1	1
				Interradial ...	2	1	1	1
5	4	4	10	Perradial	2	1	1	1
				Interradial ...	2	1	1	1
2	4	4	10	Perradial	2	1	2	1
				Interradial ...	1	1	1	1
2	4	4	10	Perradial	3	1	1	1
				Interradial ...	1	1	1	1
2	4	4	10	Perradial	2	2	1	1
				Interradial ...	1	1	1	1
2	4	4	11	Perradial	2	2	2	1
				Interradial ...	1	1	1	1
1	4	4	11	Perradial	2	2	1	1
				Interradial ...	2	1	1	1
1	4	4	11	Perradial	3	1	2	1
				Interradial ...	1	1	1	1
1	6	6	11	Perradial 0	1	1	1	1	1
				Interradial ...1	1	1	1	1	1
1	6	6	12	Perradial 1	1	1	1	1	1
				Interradial ...1	1	1	1	1	1

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Of 129 large adults taken at New Bedford in May, 1905, there were 29 variants, or 22.5 per cent. The chief features of variation are shown in the following table:

TABLE VII.

No. of Specimens.	Gastric Lobes.	Oral Lobes.	Rhopalia.	Canal System.					
1	3	3	6	Perradial	1	1	1		
				Interradial	1	1	1		
2	4	4	7	Perradial	1	1	1	1	
				Interradial	0	1	1	1	
13	4	4	9	Perradial	2	1	1	1	
				Interradial	1	1	1	1	
2	4	4	9	Perradial	1	1	1	1	
				Interradial	2	1	1	1	
1	4	4	10	Perradial	2	2	1	1	
				Interradial	1	1	1	1	
1	4	4	10	Perradial	2	1	2	1	
				Interradial	1	1	1	1	
1	4	4	10	Perradial	1	1	1	1	
				Interradial	2	1	2	1	
2	2	4	11	Perradial	2	1	2	1	
				Interradial	2	1	1	1	
2	4	4	12	Perradial	2	2	2	2	
				Interradial	1	1	1	1	
1	4	4	12	Perradial	3	2	1	1	
				Interradial	2	1	1	1	
1	6	6	11	Perradial	0	1	1	1	1	1
				Interradial	1	1	1	1	1	1
2	6	6	12	Perradial	1	1	1	1	1	1
				Interradial	1	1	1	1	1	1

I have frequently been able to confirm the observation of Bateson, (*op. cit.*), that where there are cases of marked difference in the size of the gonads of a given quadrant or of adjacent quadrants, that there is frequently a noticeable decrease in the size of the corresponding portion of the bell, as shown in Plate I, Fig. 5.

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As compared with the variations occurring in other organs, particularly the marginal organs and canals, the per cent is extremely small, as will be seen at a glance in comparing the several tables. My observations on this point confirm those of both Bateson and Browne. The former found but 1.6 per cent, while the latter found it as large as 2.4 per cent. My observations gave the average of 2.75 per cent, as the total variations to be detected in Aurelia flavidula.

As already suggested in connection with Ehrenberg's observations, in which he claimed that variation reached 10 per cent, either this must be taken to include the total, in which case it is evidently too low, or if it refer to the vegetative organs alone it is certainly too high, unless indeed it may be possible that the Aurelia aurita of the Red Sea differs very greatly from the species in other waters, or from our own species.

Rhopalia and Radial Canals.

An examination of the several tables will show that there is a general variation in the direction of an increase in the number of both rhopalia and radial canals. This has been shown to be the case in Aurelia aurita by both Ballowitz (*op. cit.*), and Browne (*op. cit.*). While confirming for the most part the results obtained by both these observers, there are points of difference which must be reviewed with some detail, and other points wherein I am unable to accept the conclusions of either in all particulars. Some of these will be considered in their appropriate connections.

Concerning the number of rhopalia little need be said further than to direct attention, as above, to the tabulated facts. A brief word or two in explanation of the Tables V to VII will suffice to render their meaning clear. Beginning with the first, or left hand column, there is listed the number of specimens having the characters given in the following columns. For example, in the second is given the number of gastric pouches, or lobes, in the third the number of oral lobes, in the fourth the number of rhopalia, and finally in the last the canal system not including the adradial system, since it bears for the most part, no direct relation to the rhopalia. The numbers following in each case refer to that of the rhopalia, and where less than the normal

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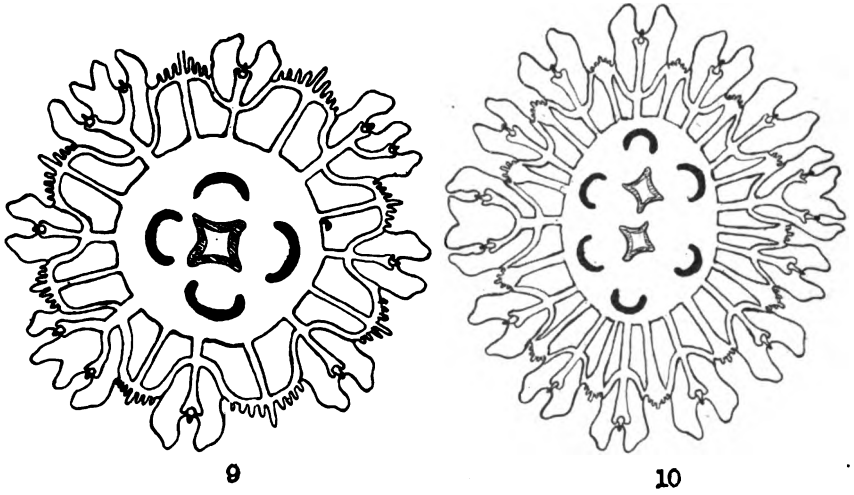
number is given, as in the first line of Table V the fact in relation to the canals is shown by the 0, indicating their absence. Where a larger number than the normal is present, as in the fifth line and those following, the figure 2 in the perradial canals indicating that the extra rhopalium is perradially located. In other words, the figures in the columns under rhopalia and canal system serve to show the correlation of the two sets of organs.

As indicated above, the tables give no account of the adradial canals, since normally they sustain no direct correlation with the



9 10

Fig. 9. Ephyra with three compound marginal lobes, quite comparable with those of Figs. 1 to 3.

Fig. 10. Ephyra with two mouths, and opposite compound marginal lobes.

other canals or with the rhopalia. It must be said, however, that there are definite exceptions to this rule. And while not sufficiently numerous to call for tabulation along with the others, they occur in too many instances to warrant the somewhat ultra pronouncement of Browne ('01, p. 100) to the contrary. Whether a given canal shall be called adradial, interradial, or perradial depends not alone on its position or whether it be branched or otherwise, but upon both its position and relations to the other canals. The name signifies nothing in itself but that of relationship. There is no intrinsic reason why an adradial canal should

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be unbranched, and as Browne admits in another connection (p. 101), there is good reason to believe that its position may shift considerably.

Figures 12, 14 and 15, are careful drawings of variant canal features, in each of which there is shown at *a* an adradial rhopalium, while in one case, Fig. 14, the canal is somewhat branched, but taken in its relations with both the other canals I see no other alternative than to regard it as adradial. This is also shown at *A*, Plate I, Fig. 2.

Both Ehrenberg and Ballowitz (*op. cit.*), have figured similar cases of undoubted adradial rhopalia.

The chief variations found in rhopalia are those of number and position, the later of which has just been noticed. The smallest number found was five, only a single specimen among the entire lot studied having so few. Six were found having but six rhopalia. Others having larger numbers are tabulated in their appropriate places in the various tables. As will be seen the largest number found was fifteen, and in but a single specimen. This specimen was further peculiar in having two fully developed and functional mouths, as shown in Fig. 10. This duplex oral condition served to give the animal a somewhat ovoid shape and at the same time a more or less bilateral aspect, the latter being further accentuated by the presence on almost exactly opposite sides of two compound marginal lobes and rhopalia, as shown in the figure.

A similar condition so far as the marginal lobes and rhopalia are concerned is shown in Fig. 9. In this case there are three compound lobes, but they do not tend in any way toward either bilateralism or even a trimerous form. An additional compound lobe would have rendered the variation a strikingly symmetrical one. But like the former and several others of a similar character which came under observation there was seldom exact symmetry.

The effect of less or more than the normal number of rhopalia may, or may not, disturb the general radial symmetry of the umbrella. For example, in Plate I, Fig. 1, is shown a medusa with but seven rhopalia, yet the general symmetry seems quite normal. By a careful inspection it is not difficult, however, to discover that one of the interradial systems is entirely lacking, Again in Plate I, Fig. 6, of a hexamerous specimen, there are

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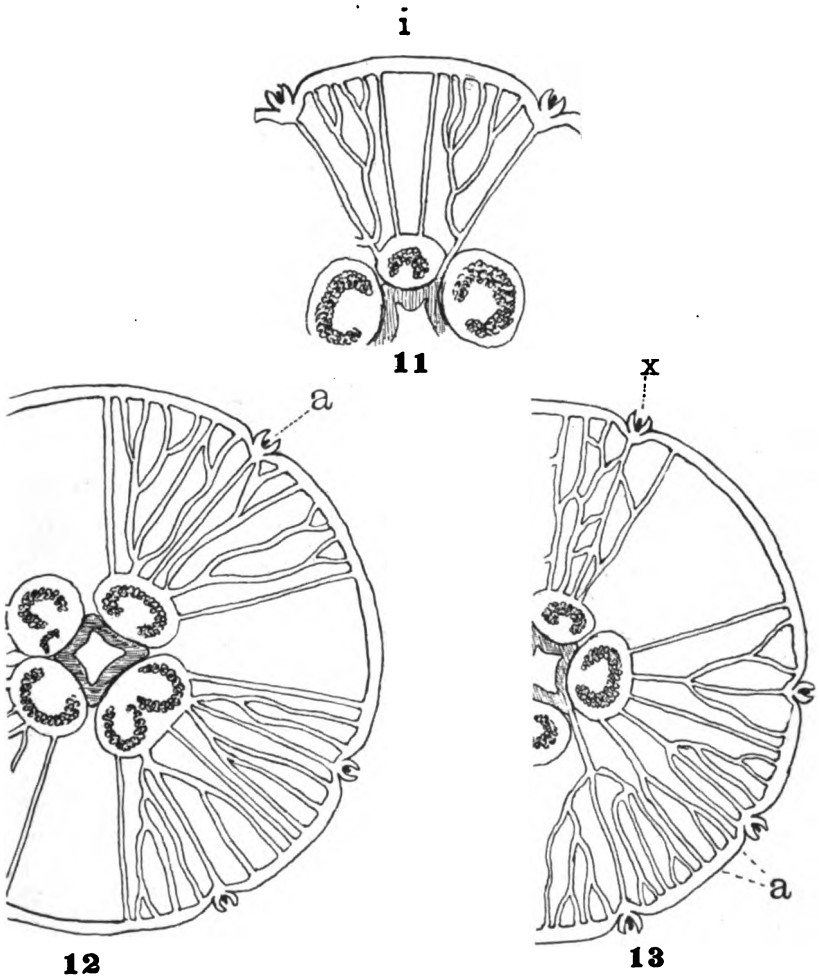

Fig. 11. Single octomere of adult medusa showing entire absence of the interradial canal, and the contiguity of two adradials.

Fig. 12. Diagram of medusa showing adradial rhopalium at *a* and lack of perradials in the octomeres drawn. Very small degenerate gonad in upper left hand pouch.

Fig. 13. Branched adradial canal at *a*, and at *x* the complete blending of the several canals of the segment into one system.

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six gonads, six oral arms, and eleven rhopalia, the twelfth being absent and its perradial canal system likewise lacking, as shown at P, and still the general symmetry is hardly affected.

On the other hand, in several of the photographs, particularly Figs. 4 and 5, it will be seen at a glance that the symmetry is more or less seriously disturbed. In still others, while the general symmetry might not appear to be seriously affected, when attention is directed to the marginal symmetry it will be seen to have suffered quite definitely, in one case three rhopalia instead of one, occupying a single octant. In such a case, which is not rare

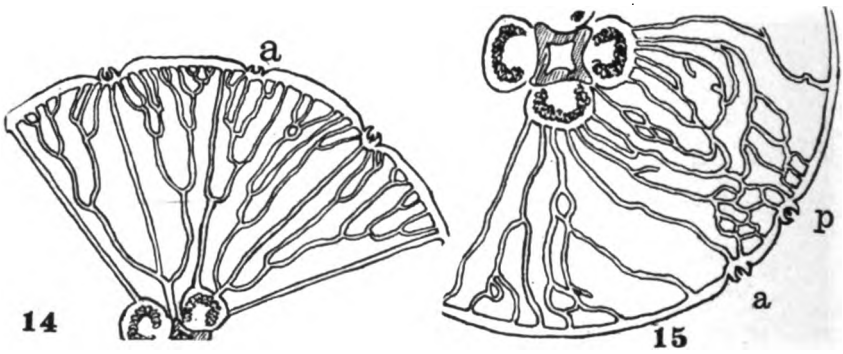

Fig. 14. Diagram showing a branched adradial canal and rhopalium shown at *a* sketched from Fig. 2 of plate.

Fig. 15. Diagram of the specimen shown in Plate I, Fig. 4, adradial canal and organ at *a*, perradial system at *p*, interradial absent or fused with the former.

the variation would seem to have been restricted wholly to that single segment, the other seven remaining normal.

And thus it is throughout; variations in one organ involving in many cases more or less distortion of the correlated organs, or even the entire organism. In other cases the variation has been associated with a regulative adjustment which has more or less served to maintain a fairly definite symmetry of both the immediate organs and the entire animal symmetry.

In connection with the study of ephyræ already given, attention was directed to the occurrence of twin rhopalia in several instances some of which are illustrated in several of the figures. Such double structures have been observed in adults, though unless

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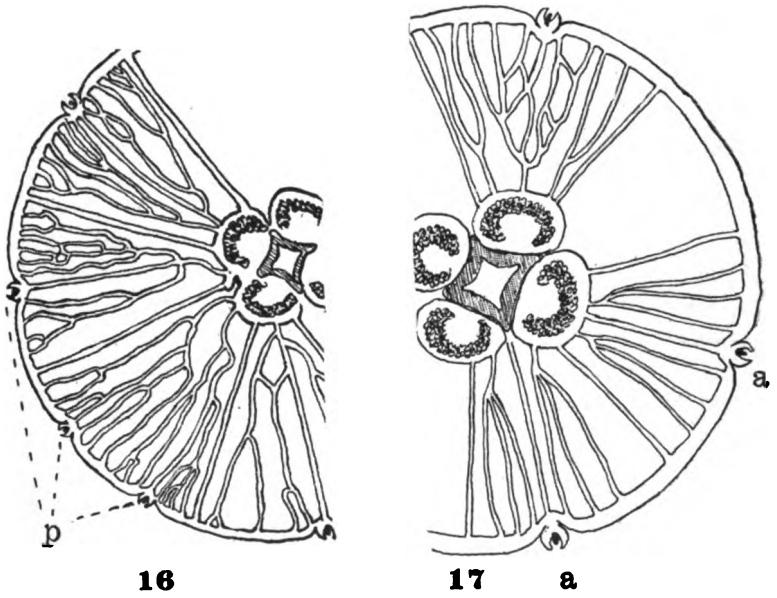
they be searched for critically they are seldom seen, since the hoods and lappets serve to screen them from ordinary observation.

It may be of some interest in this connection to refer to the occasional appearance of twin rhopalia in regeneration, an extended account of which I have elsewhere described.[1] It has been found that occasionally double rhopalia are produced in regeneration where originally there was only a single one which had been excised in the experiment. Rarely also during such experiments a super-



16 17 a

Fig. 16. Drawing showing three perradial canals and rhopalia in a given octomere, *p*.

Fig. 17. Sketch showing two octomeres *a*, *a*, with extremely simple canal systems. The positions of the rhopalia are adradial.

numerary rhopalium may develop at unusual points, as has been described in the paper just cited. It is not at all improbable, therefore, that in cases of injury the marginal portion of the umbrella involved may be more or less modified during the process of regeneration, and that marginal organs may appear in somewhat unusual places. It is not improbable that the somewhat anomalous appearance shown in Plate I, Fig. 4, may be due

[1] Regeneration in Rhizostoma pulmo. Jour. Exp. Zoöl., vol. i, p. 85.

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to regeneration following a marginal injury. A close inspection of the notch just beyond the complex network of canals will reveal the presence of a small rhopalium, indistinct in the illustration, but very distinct in the specimen. To Browne's suggestion that the absence of a marginal body may be due to injury it will suffice to have called attention to the fact that these organs are promptly regenerated and therefore would not probably be long lacking in any case in which sufficient time had elapsed for the injury to heal.

Concerning variations in the canals of Aurelia it remains to call attention to some few points not hitherto considered. In several of the photographic figures are shown varying features of anastomosis among the canals, chiefly in the peripheral portions. In addition to the case just referred to others are shown in Figs. 1 and 6. In that of Fig. 1 is shown at various points the usual type of anastomosis, while in Fig. 6 of the hexamerous specimen is shown a most complicated type affecting only one-half of the umbrella. A few other cases are figured in the diagrams in which only a single segment may be involved.

This phenomenon of anastomosis I have found about as common in the small adults as in the larger, though Browne considers it as quite rare in small specimens. I have also found it frequently affecting the terminal portion of the adradial canals, especially in those rather rare cases in which the rhopalium is adradial.

The branching of the adradial canals has already been referred to in a brief way. It is only necessary to again call attention to the matter, and refer to several of the figures in which it is shown, *e. g.*, Figs. 13, *a*, and 14, *a*. An interesting and unusual condition is shown in Fig. 11, *i*, where the interradial system is wholly lacking and the two adradials thus brought into contiguous relations.

CYANEA AND DACTYLOMETRA.

Several incidental references have been made in the preceding pages to variations observed in ephyræ of Cyanea. It has long been well known that Cyanea arctica is a remarkably variable species, so much so that Professor Agassiz recognized some of them as distinct species, and described as such Cyanea fulva, and Cyanea versicolor. But the names have long since passed into the limbo of synonomy, the forms so designated not having even a varietal recognition. In the present instance, however, they may

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serve to suggest the fact that the species varies greatly, but chiefly in the nonessentials of color and size, the southern forms being usually much smaller than those of more northern range.

In structural features I have found that this species exhibits very similar variations to those found in Aurelia. While it has not been within the scope of the present paper to enter upon any large survey of the problem as it relates to Cyanea, and no exact data have been accumulated, I have examined considerable numbers of both the ephyræ and adults, and find considerable variation in the number of rhopalia, the gastric and oral lobes, and the less important matter of coloration to which reference has been made above.

Similar observations have also been made upon our species of Dactylometra, and to the same effect. In general aspects it varies less than does Cyanea, as perhaps both vary less than does Aurelia, but concerning the fact of considerable variation there can be no doubt. In a paper upon the structure and development of Dactylometra, Mayer[1] has stated that the tertiary tentacles arise invariably on either side of the ocular lappets. While I have had no opportunity to examine any considerable number of these medusæ, I have nevertheless, found considerable disparity on this point. In several specimens examined in 1902 I found these tentacles arising at points intermediate between the primary and secondary series.

I have also found considerable variation in the number of the rhopalia and other marginal organs. Since, however, the data are too few to warrant any definite attempt at estimating the quantitative variations of either of these species, it must suffice merely to note the facts in a qualitative way and leave to another time, or other observer, the further consideration of details.

RHIZOSTOMA PULMO.

Among about fifty specimens of this medusa which I had occasion to examine critically in the progress of experiments upon regeneration, an account of which has been published elsewhere, and perhaps half as many others examined in the large aquaria less critically, about 15 per cent showed features of variation in

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one or more organs. Some of these it may be worth while to briefly consider in this connection.

As in the former series, the chief variations noted were those of the marginal organs, in which the range was from five, observed in two specimens, to twelve, found in only a single specimen; and in those of the vegetative organs—gonads, gastric and oral appendages. In these the variation was similar to the former series, though there was not close correlation, between the organs of the two series. There was, however, almost perfect correlation between the members of the same series. In other words, the gastric lobes and oral pendants were the same in number, and almost always of similar size. In a single case, and that a specimen having twelve marginal lobes and rhopalia, there was a perfect correlation of all the organs, including gastric and oral. In the usual crowding of the oral arms due to the large number present, two of these organs had been forced into the center of the group, the others forming a closely crowded circle about them. An incidental feature of two of the oral arms was that of the branching or bifurcation of the terminal portions. In the one case the lobes being unequal, while in the other they were quite uniform in size, though with the tips organically fused, or grown together. I have frequently found branched tentacles and oral arms, but it is unusual to find a subsequent union of the branches. It is rarely found in Hydra, and other hydroids, only a single case having come under my own observations.

Variations among the Rhizostomata have been recorded incidentally by several other observers, among whom are Haeckel,[1] Keller[2] and Lendenfeld.[3] The latter has given much more critical attention to the details of the problem than either of the former. This is, however, in relation to the larval, or ephyra history rather than to that of the adult. Indeed, concerning the adult this observer has recorded but a single case amony many specimens of Crambressa mosaica. Among specimens of Phyllorhiza punctata he records having noted those with more than the normal number of marginal bodies, but believes these supernumerary organs to have been a result of abnormal growth following injury.

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[1]Haeckel, E., Das System der Medusen, 1879.

[2]Keller, C., Zeits. f. wiss. Zoöl., Bd. xxxviii, S. 641.

[3]Lendenfeld, R. von., *ibid*, Bd. xlvii, S. 260, *et seq.*

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His most remarkable observations are concerned with the marginal lobes and sensory bodies of the ephyræ. He finds these to vary in number from eight, the normal, to twenty-four. While this extreme of variation is large it is not, however, improbable. The most remarkable feature of the case is the interpretation which Lendenfeld gives. He claims that during metamorphosis these larvæ pass from the ordinary condition of octamerism through stages of, first twenty-four, later sixteen and finally emerge to the adult stage with the normal eight-merous condition. "So habe ich gefunden, dass die Ephyren von Phyllorhiza punctata acht, spätere Stadien vierundzwanzig, noch spätere sechzehn und endlich die ausgebildeten Medusen wieder bloss acht Randkörper besitzen."

Though it is not expressly claimed that these phenomena are involved in the observed ontogeny of these medusæ, it is nevertheless, clearly implied. If this inference be correct then the results must be accepted unless other observations may serve to discredit the account given. If, on the other hand, as I am strongly inclined to believe, these several stages have been observed in connection with the general phases of metamorphosis such as one might find among a given series of larvæ, then the conclusion I should incline to draw is that these phenomena are but larval variations similar to those described in the earlier portions of this paper. Moreover, when we are farther advised that these peculiar variations are due to injuries the suspicion is still greater that the observed variations are not normal processes during metamorphosis, but in fact, true variations as above suggested.

Furthermore, my own experiments upon regeneration in Rhizostoma (*op. cit.*), would seem to preclude the factor of injury as of any importance in relation to variation. In these experiments there was not the slightest evidence to support the view proposed by Lendenfeld. We may, therefore, accept these instances as but further extension of our knowledge of the wide prevalence of variation among the Scyphomedusæ, which the more critical attention given to the subject during the past few years has brought to light.

FOSSIL MEDUSÆ.

It will not be without interest in this connection to call attention to the occurrence of variations among fossil medusæ. In a recent

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monograph on "Fossil Medusæ" Walcott[1] has described a large number of fossil medusæ, among which several cases of remarkable variation are recorded. In some the variation was so general as to render difficult specific diagnosis. For example, in the description of Brooksella alternata the author says: "The variation is so great in this species that a brief diagnosis is of little value." . . . "The umbrella lobes vary in number from 6 to 20 or more, and in form from broad, slightly rounded to narrow and strongly rounded. There is no regular sequence of 6, 8, 12, etc., on the contrary the irregular numbers 5 and 7 are largely represented, and 6 and 8 are abundant."

Again in his description of Laotiara cambria he says: "Its variations are greater than in Brooksella." The lobation of the exumbrella is from the simple four-lobed variety, through series of 5, 6, 7, to 8 or more, to what he designates as the compound type, which are apparently medusæ in process of fission. In this species, as in the preceding, there seems to be no definite regularity or sequence in numerical order. In the words of the author: "In many individuals there is no regularity, and in the extreme forms there is an irregular network of subumbrella lobes and oral arms."

From the numerous figures of this well-illustrated monograph it is quite evident that similar, if not equally extensive variation is also present in many other of the species described. It is not, however, within the scope of this paper to undertake an extensive review of the entire subject, and hence further details concerning this phase will not be submitted. Citation of the foregoing facts may serve to direct attention of those interested to a phase of the general problem hitherto little considered.

INFLUENCE OF ENVIRONMENT ON VARIATION.

As is well known, there is a more or less currently accepted belief in the influence of environment as a modifying factor in the variation of organisms. Reference has already been made to Haeckel's views as to the influence of such factors in relation to the abnormalities arising in medusæ reared under artificial conditions, and to his further suggestions concerning the probable influences of similar conditions in nature.

[1]Mono. Unit. States Geol. Surv., vol. xxx, Wash., 1898.

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It was with these in mind that in securing material for my investigations I endeavored to have it collected from points somewhat remote, yet in the same general region, and also from environments so definite and yet distinctly different, as to afford a means of estimating the probable effects traceable to direct and determining factors. It was an unexpected bit of good fortune that brought me into possession of material from an environment apparently quite likely to afford just the desired conditions suitable to a test. This was the occurrence of ephyræ of Aurelia in considerable numbers in April, 1902, in a small more or less isolated, and polluted pool, known at the "eel pond" located at Woods Hole, and connected with the waters of the harbor by a very small inlet, sufficient to admit tide-water daily. The pond has served in some measure as a general dumping ground for various waste and sewage from the village.

From this pond I obtained 486 ephyræ, all quite young, and many of which I was able to examine alive soon after their capture. A few specimens were obtained from strobilating polyps kept in aquaria where they thrived quite well for several weeks.

A second series was obtained from Waquoit Bay, a large bay opening directly into Vineyard Sound, and some ten miles east of Woods Hole. This collection was made in April 1901, and contained 1026 specimens.

Still a third series was collected at Waquoit in 1904, numbering about 1000. They were obtained in May, and were mostly in process of metamorphosis into young medusæ.

In 1905 a collection of adults were obtained from somewhat similar environments, one series, indeed, from Waquoit. The other numbering about 200—though on account of poor preservation only 129 were available for accurate study—were collected at the mouth of the Acushnet River, in New Bedford harbor. This environment was as unlike that of Waquoit as is the latter from the "eel pond." New Bedford harbor receives the sewage and other pollution of the city, as well as the constant influx of fresh water from the river, thus constituting an environment at once more or less local and peculiar, being about 17 miles west of Woods Hole and therefore nearly thirty miles from Waquoit.

Observations made upon the ephyræ of Cyanea during their development, a brief account of which has been given elsewhere, in which considerable variation was discovered and found to be

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due in some measure to the artificial conditions under which they were reared, led me to anticipate similar results in ephyræ obtained from an environment like the "eel pond." In this, however, I was somewhat disappointed. For while the ratio of variation found was somewhat larger than that in either of the series from Waquoit, as will be seen by a comparison of the tables, still it was far less than had been anticipated. The total number found among the 486 specimens taken in the eel pond which showed variant features was 144, or 29.6 per cent.

In the collections from Waquoit the series of 1901 comprising 1026 specimens the variants were 24.9 per cent; of the series of 1904, the 218 ephyræ showed 22.9 per cent of variants.

While the difference in favor of the eel pond series is appreciable, it is still small, too small indeed, to warrant a final conclusion as to the influence of any given factor as a determining condition. Again, it must not be overlooked that the number of specimens under consideration was likewise comparatively small.

Moreover, when we come to compare the data obtained relative to series of adults the uncertainty is greatly accentuated. Comparing the data of Table VI with those of Table VII, wherein are shown the several features of variation, it will be seen that those from the New Bedford environment, within which they were doubtless bred and reared, have a lower per cent than those from Waquoit, the exact figures of the two being 22.5 per cent for the former, and 24.3 per cent for the latter.

Therefore when a careful analysis of the available data is made we are compelled to admit that the evidence concerning the influence of environment so far as the present organisms are concerned is not convincing. And until further and more extended comparisons can be made in these or similar circumstances the answer to the general problem must be regarded as negative.

SIGNIFICANCE OF THE VARIATIONS IN RELATION TO NATURAL SELECTION.

From the foregoing review of the history of variation as it pertains to Aurelia in particular, and to a less extent to other Scyphomedusæ also, it must be quite evident that the phenomena are numerous and involve almost every part of the organism. Furthermore, so far as Aurelia is concerned, variations have been

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more or less continuous from the earliest records of von Baer and Ehrenberg to the present time precluding any probability of the operation of simply incidental factors.

An inspection of the tabulated records of more recent times will show that the tendency has been constantly toward a more or less definite increase of the several organs, particularly the marginal or sensory, though including also the central or vegetative. If one had taken occasion to construct a curve representing the various phases it would have shown that the variations had been preponderatingly upon one side of the modal line. In the absence of the curve it may facilitate a ready appreciation of the situation to submit percentage values of the variations above and below the normal.

Results obtained by Browne covering a period of about five years and including an examination of several thousands of specimens presented in percentage figures are as follows:

	Normal.	Above Normal.	Below Normal.
Rhopalia	78.71 per cent.	16.74 per cent.	4.55 per cent.
Vegetative	97.6 per cent.	1.8 per cent.	0.6 per cent.

Results obtained by my own observations covering a period of four years and an examination of about 2500 specimens are as follows:

	Normal.	Above Normal.	Below Normal.
Rhopalia	75.07 per cent.	22.97 per cent.	1.96 per cent.
Vegetative	97.24 per cent.	2.2 per cent.	0.56 per cent.

The significance of this line of rather definite and continuous variation is somewhat doubtful. Without specific details in the work of Baer and Ehrenberg it is impossible to formulate conclusions as to the ratio of variation in Aurelia aurita as observed by them, but the more recent observations of Bateson, Browne, Ballowitz and those herein described, make it perfectly certain that for at least two species of medusæ from widely separated regions variation has been remarkably active and continuous. But at the same time it seems equally certain that so far as one is able to see there has been no evidence of the operation of anything like natural selection at work. The variant forms do not appear to be more numerous than formerly, nor does the variation seem to be appreciably larger in one species than in the other, if

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indeed we really have in Aurelia aurita and Aurelia flavidula definitely distinct species, a query which has frequently forced itself upon my attention during the present research.

In this connection has naturally arisen the question as to the operation of any process akin to mutation. Mayer[1] in a recent paper on "The Variations of a Newly Arisen Species of Medusa," p. 4, reviewing the variations in Aurelia remarks: "It is evident that symmetrical "sports," or discontinuous variations of Aurelia, are continually being produced, and yet the form of the species as a whole remains unchanged."

I have previously discussed the matter of the *symmetry* of these variations, and need not take it up again, further than to say that it seems to me unfortunate to contend for the dominance of the idea of symmetry in the sense referred to, and that so far as it appears to me there is little in these variations which can be regarded as "discontinuous," or mutative. They seem on the contrary to be definitely *continuous*, and somewhat of the nature of fluctuating variations. Browne's suggestion (*op. cit.*, p. 100), that "if a very slow and gradual change is taking place in the number of tentaculocysts, then the tendency is toward the establishment of a race with ten tentaculocysts, due to an increase of two opposite perradial tentaculocysts," hardly seems warranted from the facts as known. I can hardly see that there is any such predetermined variation as would be called for by his suggestion.

So far as I am aware, the only case of variation among medusæ which might seem to be of the nature of mutation is that of Pseudoclytia pentata, Mayer (*op. cit.*). Of this case we have only the records of a single series of observations. Whether subsequent evidence will clearly confirm Mayer's conclusions remains to be seen. Furthermore, the results would have to be followed through several generations of medusæ in order to clearly establish the case as one of definite mutation, and this the future must determine.

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[1]Mayer: The Variations of a Newly Arisen Species of Medusa. Bull. Mus. Brooklyn Inst. Arts and Sciences, vol. i, 1901.

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EXPLANATION OF PLATE.

All figures were photographed natural size directly from nature.

Fig. 1. At I is shown the missing segment, of an otherwise normal specimen. Toward the periphery may be seen typical anastomoses of the radial canals.

Fig. 2. Medusa with nine rhopalia, the extra one at A occupying an adradial position, at the terminus of an adradial canal, unusual in its terminal branching.

Fig. 3. Medusa with ten rhopalia, the two extra ones occupying the same perradial segment, at P.

Fig. 4. Medusa with only seven rhopalia, one at A being at the terminus of the much curved adradial canal. At P is shown, at the margin of a complex perradial segment, a very small rhopalium. The interradial rhopalium is lacking in the adjacent segment to the left.

Fig. 5. Medusa having but three oral lobes, which were excised before the photograph was made, two of the gonads much smaller, and the umbrella of that side also appreciably narrower.

Fig. 6. Hexamerous medusæ, having eleven rhopalia, the twelfth at P with the entire perradial system of that segment lacking. On the lower right-hand side may be seen the very complex anastomoses of the canal systems of that region.

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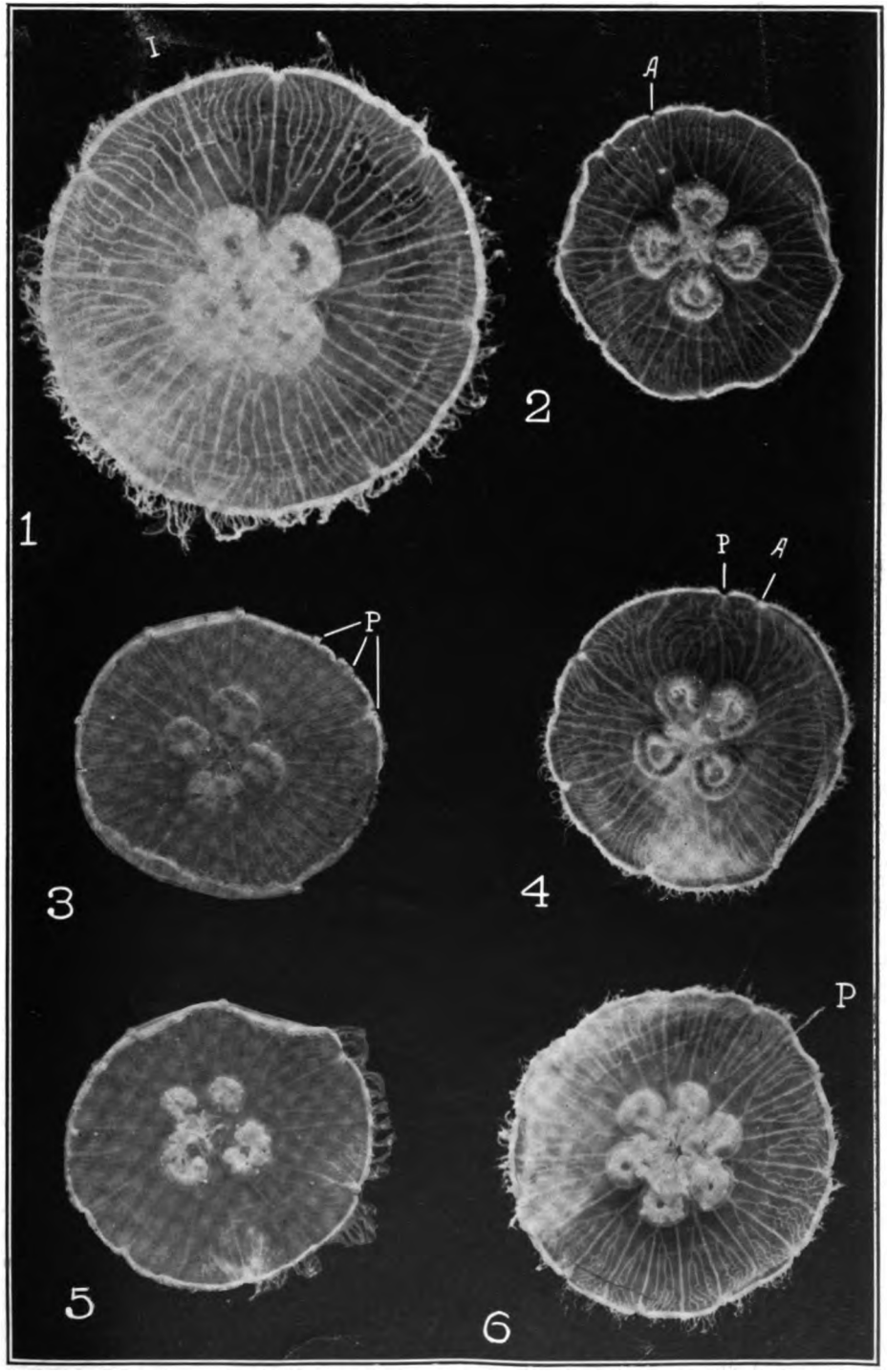

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THE ENTERON AND INTEGUMENT OF CRYPTOBRANCHUS ALLEGHENIENSIS

BY ALBERT M. REESE

WITH TWO PLATES

It was the original intention of the writer to make a detailed study of the histology of the Giant Salamander, but the unexpected appearance of much more interesting and important work has necessitated a change of plans. It has seemed best, instead of indefinitely postponing the present research, to make a less detailed study of the enteron and integument, the two regions upon which work had already been begun.

The material was collected from the tributaries of the Allegheny river, in western Pennsylvania. The chief fixing fluids used were: corrosive sublimate, corrosive-acetic mixture, Perenyi's fluid, and picro-sulphuric acid. The tissues were mostly cut in paraffin, and stained with borax carmine and Lyon's blue.

THE ENTERON

ORAL CAVITY

The lining of the oral cavity presents no particularly striking or interesting features, and a brief description of this region will suffice. The lining mucous membrane consists of a stratified epithelium of about four or five layers, the nuclei of which are large and very irregular in outline. The rest of the wall consists of connective tissue which is very dense next to the epithelium while on the side away from it it is of a much looser character and contains numerous large nuclei.

TONGUE

The tongue covers the greater part of the floor of the mouth and is supported by the well developed hyoid apparatus, from which, in

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an animal of average size, it projects freely forwards for about one centimeter. It is covered by a slightly wrinkled layer of mucous membrane. Since it is free for so short a distance from the hyoid apparatus, it has very little power of motion apart from what is given by that apparatus, and its musculature is, in consequence, not strongly developed.

A section through the mucous membrane of the tongue is shown in Pl. X, fig. 1, together with several of the small bundles of muscle fibres (*m*). The epithelium is, as might be expected, stratified, and is so deeply folded that it seems, when seen in section, to form a great number of irregular tubular glands (*g*). All of the cells of the epithelium seem capable of secreting mucous, with which they become so swollen and distended that they resemble somewhat adipose tissue, except that they retain their angular outlines. The cells of the gland-like folds, *g*, are so distended with secretion that they form a foam-like reticulum in the meshes of which a few scattered nuclei are seen. The superficial cells may show at places a fairly typical stratified structure, as at the end of the reference line *s*; at such places there is some indication of a thin cuticular border, though it is not shown in the figure.

No trace of the sense organs described by Kingsbury (94) in the tongue of *Necturus* were found, though they may have been present in other regions than those represented in the sections studied. The mucous membrane is supported by a thick corium (*c*) of comparatively dense connective tissue, composed of fine fibres with a few scattered nuclei. Through this corium are scattered blood vessels, and in its lower part are collected a few rather small bundles of muscle fibres (*m*), all of which seem to extend in the same direction. The figure shows the relatively slight development of the muscular tissue.

OESOPHAGUS

The oesophagus, which is comparatively short, is separated from the pharynx by a fairly well marked constriction, while from the stomach it is not so sharply separated. Its mucous membrane, especially in the region adjacent to the stomach, is thrown into numerous, deep, longitudinal folds. These folds, shown in Pl. X, fig. 2, are frequently spread out at the bottom into secondary folds, giving the appearance of long glands; the oesophageal glands described

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by Kingsbury (94) in *Necturus* are not present in *Cryptobranchus*.

A transverse section of the oesophagus is shown in Pl. X, fig. 2. Three chief layers may be distinguished: An outer longitudinal muscle layer (*e*), which might be called the fibro-muscular, as it is made up of rather scattered bundles of muscle fibres separated by trabeculae of connective tissue fibres; in fact this layer more resembles a very thick serosa than a layer of longitudinal muscle fibres. The second layer, lying, of course, just inside of the longitudinal muscles, is the layer of circularly arranged muscle fibres (Pl. X, fig. 2, *c*); this layer is sharply defined, and is composed of closely packed muscle fibres of the involuntary type. The third and innermost layer is the mucosa (*m*), composed of a lining epithelium supported by a comparatively thick mass of connective tissue.

The mucosa is the only layer that need be described in any further detail. The lining epithelium of the mucosa is of the ciliated stratified variety, instead of the simple ciliated columnar type that is described in *Necturus* by Kingsbury. It is thrown into deep folds (Pl. X, fig. 2) to conform to the longitudinal folds of the mucosa that have already been mentioned. Three kinds of cells may be distinguished, ciliated columnar, goblet, and irregular basal cells (Pl. X, fig. 3). The columnar cells (Pl. X, fig. 3, *c*) are irregularly pyramidal in shape, the narrow base of the pyramid being the exposed side which is covered with rather short, fine cilia. The nucleus is large, coarsely granular, and lies in about the middle of the cell, which it bulges out at that place. The pointed end of the cell projects down among the lower cells, so that it is difficult to determine its outline. The cytoplasm is finely granular. The goblet cells (Pl. X, fig. 3, *b*) vary in number in different regions, being sometimes almost wanting, and again, being quite as numerous as the ciliated columnar cells just described. They vary in shape with the amount of secretion they contain; they have the appearance of being columnar cells which have become distended, beginning at the free end, with a clear secretion. The cell figured is a typical goblet cell of this region of the enteron. It shows the free end of the cell enlarged by the contained mass of secretion, while the lower or middle part of the cell is bulged out by the nucleus, as in the ordinary columnar cell. Between the nucleus and the terminal mass of secretion is small mass of protoplasm that still shows a granular structure. The irregular basal cells (Pl. X, fig. 3, *a*) have no defi-

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nite shape; they are usually nearly filled by their contained nuclei, and are apparently arranged in about two rows.

The tunica propria, or layer of connective tissue that supports the epithelium, consists of a fairly compact mass of fine fibres with numerous nuclei and small blood vessels. The muscularis mucosa, if present, is represented by such scattered fibres that it cannot be determined; there is, therefore, no distinction between the tunica propria and the usual submucosa.

STOMACH

There is no externally visible line of demarkation between the stomach and the oesophagus. When empty the stomach is an elongated organ whose walls, externally, are rather smooth and fibrous, while internally they are thrown into many complicated folds.

The usual layers of the stomach may be made out, and are shown in Pl. X, fig. 4. The fibrous or serous coat can hardly be said to exist as a distinct layer, though the outer or longitudinal muscular layer (*l*) is often made up of almost as much fibrous as muscular tissue, and might be called the fibro-muscular layer: this layer is of about the same thickness throughout the stomach. The circular layer of muscles (*c*) is much more strongly developed than the longitudinal, and increases markedly in thickness in passing from the cardiac to the pyloric end. The muscular coats are, of course, composed of involuntary fibres. The submucous coat (*sm*), is of the usual character, and is well developed: it becomes somewhat thinner towards the pyloric end of the stomach. The muscularis mucosa (*mm*) is fairly well developed: it shows an indistinct division into an outer longitudinal and an inner circular layer (this is not indicated in the figure).

It is in the structure of the mucous membrane that the chief differences between the two ends of the stomach are seen. The cardiac mucosa will first be described. It consists of a single layer of columnar epithelium (Pl. X, fig. 4, *e*), overlying a fibrous tunica propria (*m*) and dipping down into the cardiac glands (*g*); the muscularis mucosa has already been mentioned. The epithelium (Pl. X, fig. 5, *e*), on the free surface, consists of tall, columnar cells whose outer ends are more or less clear, while the inner ends are finely granular. The nuclei are large, and lie in the lower half of

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the cells, in some cases, in other cases in the middle of the cells. The cardiac glands are of the usual compound tubular character; they are moderately long, and are not nearly so closely aggregated as in the higher animals (Pl. X, fig. 4). Each gland (Pl. X, fig. 5) may be divided into three regions; the neck or duct (*n*), the mucous region (*s*), and the fundus (*f*). The duct or neck is comparatively wide and may form half the entire length of the gland; it is lined with the same cells as those that have been described as covering the free surface of the mucous membrane. Two, or possibly three, ducts may unite to form one opening to the surface. Below the neck the gland is considerably enlarged by a collection of clear, mucous cells, shown rather diagrammatically in the figure at *s*; this is the mucous region. These mucous regions which are more strikingly marked off from the rest of the glands than is indicated in the figure form a very characteristic feature of the cardiac end of the stomach. The mucous cells are large and irregular in shape (not well shown in the figure); each cell contains, near its base, a nucleus which is smaller and more rounded than those of the neck region. The swollen, globular character of the mucous region is much more marked in the form under discussion than in *Necturus* as described by Kingsbury. The deeper or fundic region (Pl. X, fig. 5, *f*, *f'*) is elongated, convoluted, and frequently branched; it is narrower than the rest of the gland, and, owing to its convolutions, is seldom cut through its entire length by any single section. The cells of this region are irregularly cuboidal in shape, and contain nuclei of the same general character as those of the mucous region. The cells are filled with granules which are somewhat coarser than those of the neck region, but no distinction into a clear outer and a granular inner zone is to be made out in the preparations at hand.

The mucous membrane of the pyloric end of the stomach (Pl. X, fig. 6) is much thinner than that of the cardiac region, and differs from it markedly in structure; there is, however, no sharp line of demarkation between the two regions, the cardiac glands changing gradually to the form characteristic of the pyloric region. The entire pyloric gland practically corresponds to the neck region of the cardiac gland. Near its bottom it narrows suddenly, and sometimes branches; these branched portions are always short. The epithelium is of the tall columnar variety; the nuclei are located in the bases of the cells, where the small amount of granular proto-

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plasm is collected. The outer half of the cells (*e*) is clear and mucigenous; the free ends being rounded and often constricted to form a sort of knob, as shown at *e*. This clear zone is much deeper and more strongly marked in this region than in the cardiac portion of the stomach, though it is not very distinct in the narrow, branched portion of the glands (*f*). The fibrous tunica propria that supports the epithelium and glands (*m*) is of the same character as in the cardiac region, but is, of course, thinner.

INTESTINE

While the stomach, as has been said, is not sharply marked off from the oesophagus, it is sharply differentiated, even externally, from the intestine by a well marked constriction. The intestine is quite narrow, though its diameter varies considerably, and, in an animal of average size, is about thirty-five centimeters in length. Externally the intestine has the usual smooth appearance, while internally it is thrown into numerous longitudinal folds (Pl. XI, fig. 7). The size of the lumen varies greatly, being sometimes reduced to a very narrow passage (Pl. XI, fig. 7), while again it may be surprisingly wide.

Pl. XI, fig. 7 is a camera drawing of a transverse section to show the relative thickness of the various layers and the irregular outline of the lumen. The outermost layer (Pl. XI, fig. 8, *s*) is a very thin fibrous coat, so closely adherent to the outer muscular layer that it is easily overlooked. The longitudinal and circular muscular layers (Pl. XI, figs. 7 and 8, *l* and *c*) are of about equal thickness, the former being composed of scattered muscular fibres among which are numerous connective tissue fibres and blood vessels, and the latter of a compact mass of circularly arranged muscle fibres. The submucosa has the usual appearance of that layer; it is thrown into the deep longitudinal folds that have already been mentioned (Pl. XI, figs. 7 and 8). The epithelium is of the simple columnar type (Pl. XI, figs. 8 and 9), and follows, of course, all the foldings of the fibrous layer against which it lies. A top-plate or striated border (*t*) is very distinct. Two kinds of cells may be recognized; the typical columnar cells, and the goblet cells. The former (Pl XI, fig. 8, *e*, and fig. 9, *a* and *b*) vary considerably in width and shape according to their location; on the sides of the folds they are quite regularly columnar, while those at the top of the folds are narrow

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at the inner end and wider at the outer, and those at the bottom of the folds are compressed at the free end and wide at the inner end. Each cell is finely granular, and contains a large oval nucleus which lies about in the middle of the regular cells, and towards the broader end of the pointed cells. The goblet cells (Pl. XI, fig. 8, *g*, and fig. 9, *c* and *d*) vary considerably in number and size; the nucleus lies near the basal end, though its position varies in different cells. All stages may be seen, from the columnar cell that is just beginning to become clear and swollen at its outer end, to the fully formed, typical goblet cell.

The glands described by Kingsbury in *Necturus* seem not to be present. They may be represented by certain rather indefinite groups of cells that are occasionally found at the lower angles of the folds (Pl. XI, fig. 8, *n*); no indication of a lumen is to be seen in any of these groups of cells, so that it seems more likely that they are, as has been suggested by Bizzozro and Nicolas for other forms, simply groups of young cells from which new epithelial cells are derived.

RECTUM

The intestine, after a slight constriction, merges suddenly into the cloaca or rectum, which is about eight centimeters long in an individual of average length. It is of considerably larger diameter than the intestine, and is sometimes of about equal diameter throughout, sometimes narrower towards the posterior end.

Disregarding the marked folds of the intestine which are not present in the rectum, the walls of the latter region are somewhat thicker than those of the former. The longitudinal folds being absent, as has been said, the lumen of the rectum is much wider than that of the intestine. The serous, muscular, and submucosal layers are about the same as in the intestine, and need not be further described (Pl. XI, fig. 10). The mucosa, which is comparatively smooth because of the evenness of the submucosa, is a stratified epithelium. The superficial cells are of two kinds; irregular, tall columnar cells, and goblet cells (Pl. XI, fig. 11). A marked striated border is present as in the intestine (Pl. XI, fig. 10). The nuclei of the columnar as well as of the goblet cells are oval in shape and situated near the base of the cells. Beneath the superficial layer of columnar and goblet cells are two or three layers of short, irregular cells (Pl. XI, fig. 10, *i*) whose outlines are difficult to determine: each of these cells contains a large round nucleus.

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THE INTEGUMENT

The surface appearance of the skin is one of the most characteristic features of the Giant Salamander. The two lateral folds, extending from the front legs to a point some distance back of the posterior limbs, are very striking, and add to the animal's unprepossessing appearance. The general color is a greenish-brown, with dark, irregularly scattered blotches. The color varies in the same individual, being sometimes nearly black, and sometimes of a decided reddish tinge; this change in color takes place in both sexes.

The skin, like that of most of the amphibia, is smooth and slimy, although the slime is not so abundant as in some other forms. The animals, when kept in captivity, shed from the entire body a thin, transparent cuticle, which may be preserved in formalin and used as an excellent demonstration of pavement epithelium, for classes in histology (Pl. XI, fig. 12).

Scattered over practically the entire body, but especially over the head, are numerous small, light-colored papillae, the neuromasts or organs of the lateral line system; their arrangement has been described by Malbranch (76) and Kingsbury (95). Each papilla is about the size of a small pin-head, and is either rounded or oval in shape (Pl. XI, fig. 13, *n*).

A small portion of the integument from the top of the head is represented in Pl. XI, fig. 13. It was cleaned of the underlying connective tissue, dehydrated, cleared, and drawn under a magnification of forty diameters. It shows, in the centre, an oblong papilla (*n*), with a dozen or more glands (*g*) grouped about it. The neuromast shows a slight transverse furrow, but no duct or opening. Nearly all of the glands, on the other hand, show a distinct, and generally elongated, opening (*d*). Scattered among the glands and neuromasts are numerous, black, pigment spots (*p*), which show a tendency to arrange themselves around the glands. There are also numerous brown, finely granular spots among the more striking black spots. It may be a variation in the relative numbers of these two kinds of spots that causes the change in color that has been mentioned. In a portion of the skin viewed, as in the figure, by transmitted light, the glands stand out very markedly as clear circles in a darker background; and while they are not evenly distributed, they are usually very close together, so that they are present in enormous numbers.

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A section of the integument, passing through a single neuromast and two glands, is shown in Pl. XI, fig. 14. Three regions may be distinguished; an epidermal layer (*e*), a fibrous layer (*f*), and a muscular layer (*m*). The epidermis varies considerably in thickness in different regions; it shows practically no differentiation into layers, except that the cells on the free surface, next to the cuticle, are slightly flattened. In some regions the cells are irregularly cuboidal, in other regions they are somewhat elongated in a direction perpendicular to the surface. The cuticle (*c*) scarcely shows in sections, except where it is slightly separated from the underlying cells. The fibrous or connective tissue layer is made up of a loose mass of fibres, through which are scattered the large, black pigment cells (*p*) that have already been mentioned. Into this layer project the glands and neuromasts from the epithelial layer. The muscular layer varies somewhat in thickness in different regions. and is made up of a rather definite mass of intertwining fibres.

The glands are either of two distinct kinds, or they vary in appearance in the different stages of their activity. At *g* is shown a gland that is cut through the centre, showing its opening to the surface (*d*). This gland, which happens to be empty of secretion, shows the layer of irregular cells (*s*) that form its outline, and the funnel-shaped opening through the epidermis. The cells of the glandular wall seem to project into the mouth of this funnel, and are, at this place, two or three layers in depth (*t*); Schneider (02) calls these cells, that really form the beginning of the duct, the "intercalary cells." The cuticle passes down into the duct, between the intercalary cells and the epidermis. Surrounding the epithelium of the gland is a fairly distinct basement membrane, but the muscular layer that is sometimes described cannot be made out. The nuclei of the glandular cells are small and irregularly oval in shape. A second gland is shown at *g'*. This gland is cut through one side, so that it seems smaller than the other, and does not show its duct. The secretion with which this gland is filled is granular in appearance, and takes stains readily, so that it always has a decided color. The secretion of the gland first described, on the other hand, is very slightly affected by stains, and is not so markedly granular in appearance. The nuclei of the second kind of gland are less numerous than those of the first, and are considerably larger and more rounded in shape.

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The neuromast that is represented in Fig. 14 is cut in a plane at right angles to its long axis. It does not show as marked an elevation above the general surface of the skin as might be expected. It will be noticed that the epidermis dips down, and, becoming reduced to a single layer of cells, forms the outer wall of the neuromast. A comparatively wide opening connects the cavity of the organ with the exterior. The structure of the neuromast is essentially the same as that described by Schneider (02) and Kingsbury (95) for other Urodela. The main body of the organ is made up of elongated supporting cells (*r*), whose nuclei form a more or less regular row at the outer ends. The elongated cytoplasmic portions of these cells do not stain readily, so that it is difficult to determine accurately the cell boundaries. At the bottom of the cavity of the neuromast, among the inner ends of the central supporting cells, is a small group of cells (*o*) which doubtless correspond to the "sensory cells" of other workers. They are small, conical cells, with deeply-staining nuclei. Their pointed ends often project slightly into the cavity of the neuromast. The nervous supply of the organ cannot, with the material at hand, be definitely determined, but a rather indefinite mass of fibres and cells (*n*) extending from the bottom of the neuromast may indicate the position of the entering nerve.

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EXPLANATION OF PLATES

Plate X

Fig. 1. Vertical section through the dorsal surface of the tongue: *c*, sub-epithelial connective tissue, containing many blood vessels; *g*, glandular foldings of the epithelium; *m*, bundles of muscle fibres; *s*, ordinary columnar epithelium; *s'*, irregular, swollen epithelial cells at the opening of one of the glands.

Fig. 2. Transverse section through the well of the oesophagus: *c*, circular muscle fibres; *e*, epithelium; *l*, layer of longitudinal muscle fibres; *m*, layer of sub-epithelial connective tissue.

Fig. 3. Epithelium of the oesophagus: *a*, section through the entire thickness of the epithelium; *b*, a single goblet cell; *c*, a single columnar cell.

Fig. 4. Transverse section through the wall of the cardiac end of the stomach, to show the general arrangement of the different layers: *c*, layer of circular muscle fibres; *e*, epithelium; *g*, cardiac glands; *l*, longitudinal layer of muscle fibres—the thin outer border of this layer represents the fibrous or serous layer; *m*, fibrous tunica propria; *mm*, muscularis mucosa; *sm*, submucosa.

Fig. 5. Vertical section through the mucous membrane of the cardiac region of the stomach, showing the structure of the cardiac glands: *e*, superficial epithelium; *f*, fundus of a gland, cut longitudinally; *f'*, transverse section through the fundus of a gland; *m*, tunica propria; *n*, duct of the gland; *s*, swollen mucous cells.

Fig. 6. Vertical section through two glands of the pyloric region of the stomach: *e*, epithelium, composed of a single layer of columnar cells with a clear outer and a granular inner zone, the latter containing the nucleus, the outer zone being, in many cells, constricted to form a sort of knob-like end; *f*, narrow fundus of gland, cut transversely; *m*, fibrous tunica propria; *n*, open neck of gland.

Plate XI

Fig. 7. Transverse section through the entire intestine, to show the relative thickness of the different layers and their general arrangement: *c*, circular muscular layer; *d*, lumen of the intestine; *l*, longitudinal layer of muscle; *m*, fold of the mucous membrane; *s*, mesentery.

Fig. 8. Transverse section through a portion of the intestinal wall, to show the details of structure in two of the longitudinal folds: *a*, blood vessels; *c*, circular muscular layer; *e*, epithelium; *g*, goblet cell; *l*, longitudinal muscle layer; *n*, group of young cells; *s*, serosa; *sm*, submucosa; *t*, top-plate or striated border.

Fig. 9. Isolated cells from the epithelium of the intestine: *a* and *b*, columnar cells; *c* and *d*, goblet cells.

Fig. 10. Transverse section through the wall of the rectum or cloaca: *c*, circular muscle layer; *e*, superficial layer of columnar cells of the mucous membrane; *i*, deeper layers of rounded cells of the epithelium; *l*, longitudinal muscle layer; *s*, serosa; *sm*, submucosa.

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Fig. 11. Isolated cells of the rectum: *a* and *b*, columnar cells; *c*, goblet cell.

Fig. 12. Surface view of cuticle of integument.

Fig. 13. Surface view of the integument as seen under the low power of the microscope; cleared in xylol and viewed as a transparent object: *d*, opening of a gland; *g*, a gland; *n*, an oblong papilla; *p*, pigment spot.

Fig. 14. Vertical section of the integument, passing through a single neuromast and two glands: *c*, cuticle, represented in surface view in Fig. 12; *d*, duct of larger gland; *e*, stratified epithelium; *f*, fibrous supepithelial layer; *g*, a large gland; *g'*, a small gland; *m*, layer of muscle fibres; *n*, nerve (probably) to neuromast; *o*, sensory cells of neuromast; *p*, pigment cells; *r*, supporting cells of neuromast; *s*, cells of the wall of the gland; *t*, "intercalary cells" of gland.

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PLATE X

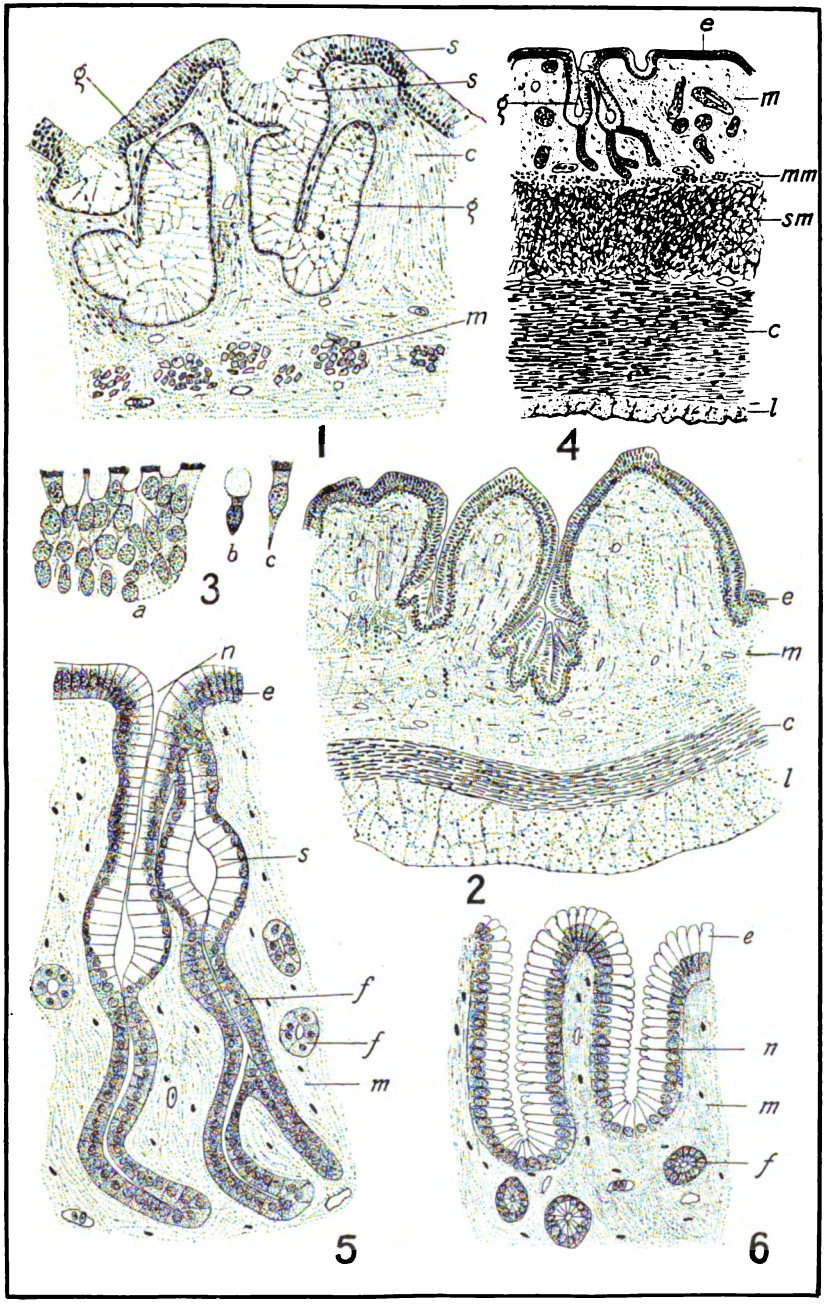

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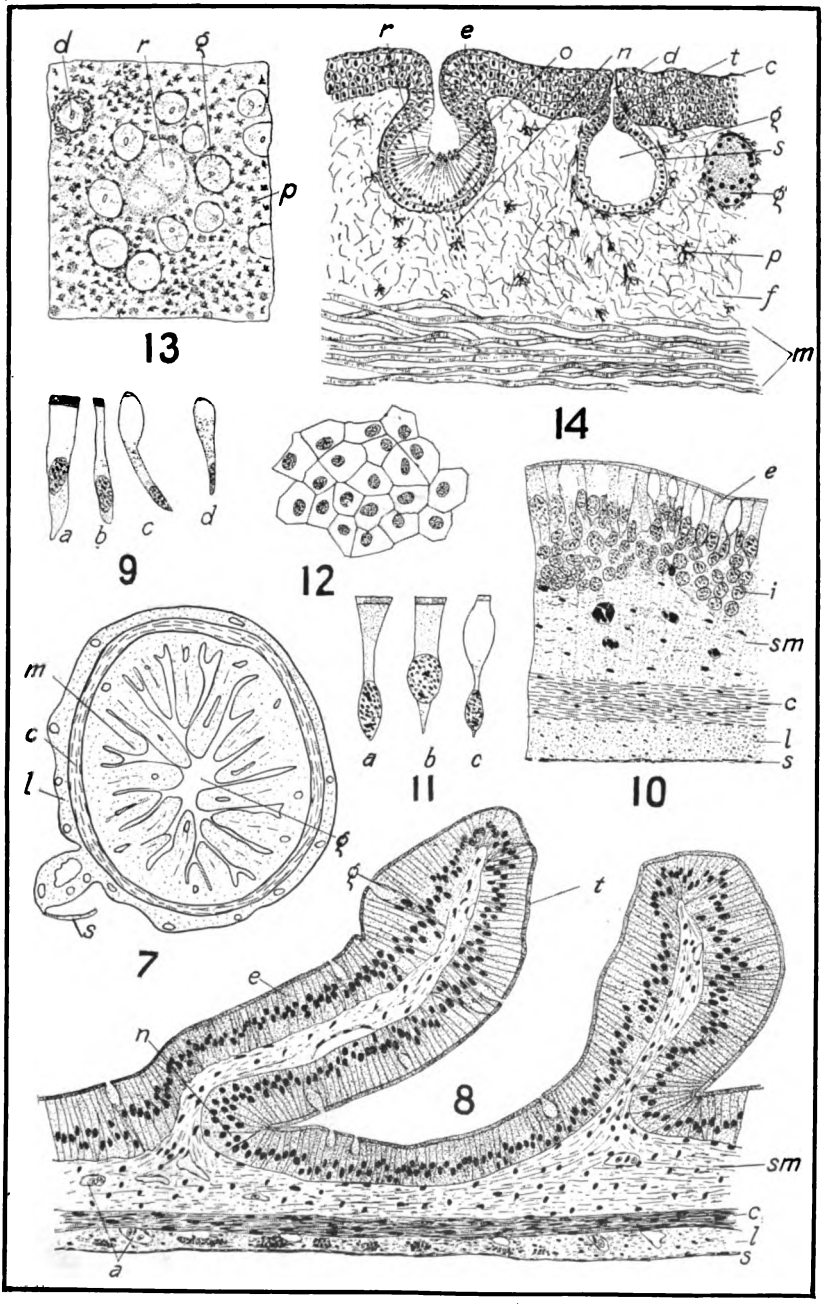
PLATE XI



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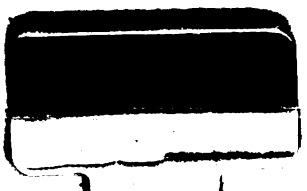

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